

Ulla Hedner

On Fibrinolysis

- A. Fibrin/fibrinogen degradation products
with special reference to renal diseases
- B. Inhibitors of activation of plasminogen

Malmö 1974



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This survey is based on the following papers:

- A. I. Bouma, B.N., Hedner, U. and Nilsson, I.M.:
Typing of fibrinogen degradation products
in urine in various clinical disorders.
Scand J clin Lab Invest 27:331, 1971.
- II. Hedner, U.: Urinary fibrin/fibrinogen degradation products (FDP) in renal diseases and during thrombolytic therapy. Scand J clin Lab Invest. In press.
- III. Hedner, U., Ekberg, M. and Nilsson, I.M.:
Urinary fibrin/fibrinogen degradation products (FDP) and glomerulonephritis. Acta med scand. In press.
- B. IV. Hedner, U. and Nilsson, I.M.: Urokinase inhibitors in serum in a clinical series. Acta med scand 189:185, 1971.
- V. Hedner, U., Nilsson, I.M. and Jacobsen, C.D.:
Demonstration of low content of fibrinolytic inhibitors in individuals with high fibrinolytic capacity. Scand J clin Lab Invest 25:329, 1970.
- VI. Hedner, U. and Åstedt, B.: Studies on fibrinolytic inhibitors during pregnancy. Acta obstet gynec scand 50:99, 1971.
- VII. Pandolfi, M., Hedner, U. and Nilsson, I.M.:
Bilateral occlusion of the retinal veins in a patient with abnormally large content of inhibitors of fibrinolysis in the blood. Ann Ophthal 2:481, 1970.
- VIII. Hedner, U.: Studies on an inhibitor of plasminogen activation in human serum. Thromb Diath haemorrh. In press.
- IX. Hedner, U. and Nilsson, I.M.: Pattern of the activator inhibitor in carcinoma and renal disease. Scand J Haemat. In press.

The articles will be referred to in the text by their Roman numerals.

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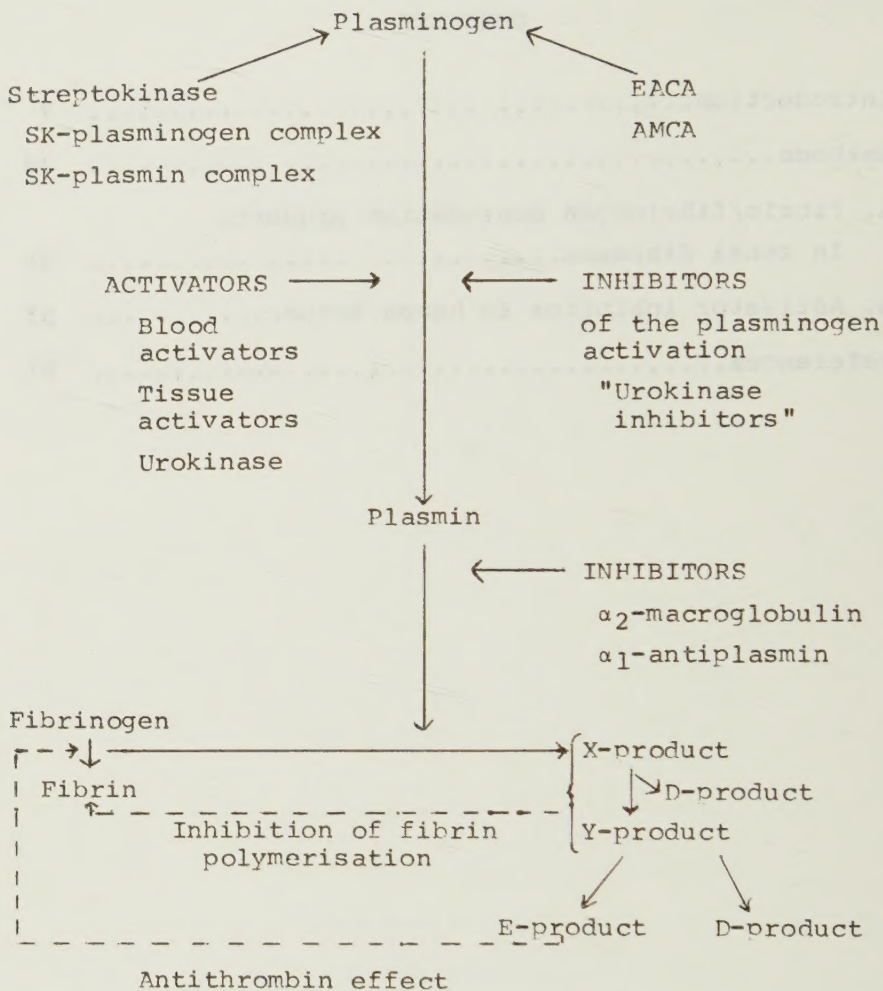


Fig. 1 The fibrinolytic system

INTRODUCTION

Fibrinolysis is an enzymatic process in which the proteolytic enzyme plasmin breaks down fibrinogen and fibrin. A diagrammatic representation of the fibrinolytic system is given in Fig. 1. The blood contains the inactive enzyme precursor plasminogen, which is activated to the active proteolytic enzyme plasmin under the influence of various activators. The plasmin breaks down fibrinogen and fibrin into fibrin/fibrinogen degradation products (FDP) of different molecular sizes, high molecular weight degradation products (X- and Y-products) and low molecular weight degradation products (D- and E-products). The conversion of plasminogen to plasmin can be inhibited by various inhibitors of plasminogen activation. The action of plasmin can be inhibited by antiplasmin, immediately reacting as well as progressively reacting.

Plasminogen is a β_2 -globulin (Oncley et al 1959, Derechin et al 1962, Robbins and Summariia 1963, Robbins et al 1965, Ganrot & Niléhn 1968). It has been shown by Christensen & Smith (1950) that plasminogen is soluble and stable in acid solution. This was utilised in Kline's method for purifying plasminogen (Kline 1953, Kline & Fishman 1961b). The solubility at neutral pH was, however, low after acid treatment. It was shown by Wallén & Bergström (1960) and Bergström et al (1971) that addition of EACA or lysine increased the solubility of the plasminogen molecule; this has been utilised in the purification of plasminogen (Wallén & Bergström 1960, Mosesson 1962, Robbins et al 1965, Bergström et al 1971). Affinity chromatography with lysine coupled to Sepharose or polyacrylamide and with the use of EACA in the elution buffer has recently been devised for the purification (Deutsch & Mertz 1970, Rickli & Cuendet 1971, Hatton & Regoeczi 1973).

E-aminocaproic acid (EACA) as well as tranexamic acid (AMCA) induce conformational changes in the plasminogen molecule (Alkjaersig et al 1959a, Abiko et al 1969, Johnson et al 1969b, Brockway & Castellino 1972, Wallén & Wiman 1972), as judged from a decrease in the sedimentation constant and an increase in molecular weight. Lysine has a corresponding effect on the plasminogen molecule (Brockway & Castellino 1972).

The molecular weight has been found to be 81,000 (Barlow et al 1969, Summaria et al 1973), 86,000 (Wiman & Wallén 1973) and 89,000 (Robbins et al 1965, Rickli & Otavsky 1973). In 1966 Johnson et al assumed the existence of multiple forms of plasminogen in human plasma. This assumption was confirmed by Wallén & Wiman (1970, 1972) and Collen et al (1972a), who demonstrated 6 forms, indicated by different electrophoretic properties and isoelectric points, which ranged from 6.0 to 6.6. Hatton & Regoeczi (1973) also produced evidence that circulating plasminogen consists of iso-enzymes. An extensive study of the physico-chemical properties of the different iso-enzymes has recently been presented by Summaria et al (1973). The molecular weight of these fractions is 90,000, as determined by gel filtration (Wallén & Wiman 1972) or 80,000 determined by ultracentrifuge (Summaria et al 1973). The plasminogen molecule is a single chain monomer with 21 to 22 disulphide bonds (Robbins et al 1967). According to Wallén & Wiman (1972), purified preparations of human plasminogen often contain not only those components normally occurring in plasma, but also components with abnormal electrophoretic mobilities and with isoelectric points varying between 7.3 and 8.8. The fraction containing these abnormal components was found to have a molecular weight of 105,000. The authors felt that these components were due to partial proteolytic degradation of the plasminogen molecules, followed by conformational changes. The isoelectric point shifted from pH 6 to about pH 8 on activation of plasminogen to plasmin (Wiman & Wallén 1973). The components occurring in normal plasma (Wallén & Wiman 1972) were constantly found to have glutamic acid in the N-terminal position and a homogeneous sequence of the N-terminal hexapeptide. The N-terminal amino acids of the partially degraded fragments varied and consisted predominantly of valine and lysine. Proteolytic degradation of the plasminogen molecule thus seems to split off peptides from the N-terminal part of the molecule (Wiman 1972), which indicates that one of the sites of autocatalytic digestion of plasminogen, described by Alkjaersig et

al (1958a), is situated in the N-terminal fragment (Wiman 1972). After cleavage of the plasminogen molecule by cyanogen bromide Wiman (1972) isolated a N-terminal fragment with a molecular weight of 7,000; this fragment was acidic. Further studies by Wiman & Wallén (1973) have confirmed the splitting off of a low molecular weight fragment (mol.wt 8,000) from the plasminogen molecule resulting in an inactive intermediate compound with a mol.wt of 86,000. This step was accompanied by an increase in methionine as a N-terminal amino acid. During the further degradation (step 2) valine and lysine became the predominant N-terminal amino acids. Active plasmin was found during step 2 and the intermediate compound was split into two polypeptides (mol.wt 63,000 and 25,000) connected by disulphide bonds. Essentially the same finding was made by Rickli & Otavsky (1973) and Claeys et al (1973). As pointed out by Collen (1973), the plasminogen with a molecular weight of 86,000 and N-terminal acids lysine or valine has a much shorter half-life ($T/2$: 0.7-1.0 day) than the plasminogen with a mol.wt of 90,000 and glutamine as the N-terminal acid ($T/2$: 2.0-2.5 days).

Plasminogen as well as plasmin has a high affinity for fibrinogen and fibrin (Müllertz 1953a, Alkjaersig et al 1959b). Fibrinogen preparations thus usually contain plasminogen (Lassen 1952, Vignal et al 1962, Brakman 1965). The intrinsic clot lysis theory presented by Alkjaersig et al (1959b) is based on the assumption that the amount of plasminogen entrapped in the fibrin clots and thrombi is sufficient to promote lysis of the clot after the plasminogen has been activated to plasmin by circulating activator (Sawyer et al 1961). It has, however, been shown by several authors that serum and plasma contain equal amounts of plasminogen (Fantl 1962, Hedner & Nilsson 1965, Ogston et al 1966). An immunofluorescent method has shown that retracted clots and thrombi do not contain plasminogen (Gottlob et al 1973). The same author showed that thrombi older than 48 hours, which had undergone swelling, contained more plasminogen than the blood.

The site of production of plasminogen is still uncertain. Barnhart & Riddle (1963) suggested the eosinophilic leukocytes as a possible site. But a more probable site is the liver cells (Sherry 1968, Nilsson 1971). The amount of plasminogen in plasma has been given as 200 $\mu\text{g/ml}$ (Sherry 1968, Rabiner et al 1969, Collen et al 1972a) and the half-life of plasminogen as 2.21 ± 0.29 days (Collen et al 1972a) or 2.81 ± 0.24

days (Takeda 1972). A congenital decreased level of plasminogen has been described in some families by Jacobsen (1968).

Plasmin. Activation of plasminogen to plasmin by urokinase or streptokinase involves the cleavage of a single arginyl-valine bond, which results in a two-chain molecule, in which the chains are connected by a single disulphide bond (Robbins et al 1967, Summaria et al 1967a, Barlow et al 1969, Robbins & Summaria 1971).

The molecular weight of this two-chain molecule is 75,400, suggesting that a peptide is split off during activation (Barlow et al 1969, Robbins & Summaria 1971). The cleavage of the single disulphide bond results in complete loss of activity with the formation of two chains (Summaria et al 1967a, 1971a), a heavy one (mol.wt 48,800) and a light one (mol.wt 25,700). The β -chain (light) occur in at least three different forms with varying isoelectric points in urokinase activated plasmin (Robbins & Summaria 1973). The single proteolytic site of the plasmin molecule containing a serine and a histidine residue (Summaria et al 1967b, Groskopf et al 1969, Robbins et al 1973) is located in the light chain, which corresponds to the carboxyl-terminal portion of the molecule. The sequences of amino acids around the active centers, serine and histidine residues, have been determined (Groskopf et al 1969, Robbins et al 1973). Alpha-amino substituted lysine and arginine esters are specific substrates for the plasmin (Troll et al 1954) and recently plasmin has been found to split the lysyl-lysine and lysine-arginine bonds of the fibrin(ogen) molecule (Robbins & Summaria 1971). Plasmin degradation of fibrin(ogen) results in the formation of fibrin/fibrinogen degradation products (FDP) of different types.

Plasmin is a fairly non-specific proteolytic enzyme and attacks a number of other plasma proteins, such as the coagulation factors V and VIII (Sherry 1968, Pasquini & Hershgold 1973). The non-specificity of the enzyme has been utilised in assay systems for plasmin. Thus, proteins, such as casein (Remmert & Cohen 1949, Hedner & Nilsson 1965, Lauritsen 1968, Johnson et al 1969a) and gelatine (Kaplan 1944, Christensen 1945) have been used as substrates in methods for determining plasmin.

Activators. The activation of plasminogen is effected

by different naturally occurring activators viz a) tissue activator, b) blood activator and c) urine activator (urokinase).

Tissue activator. It was shown by Astrup & Permin (1947) that the fibrinolytic activity of tissues is due to an activator which is of cellular origin and not dependent on the life of the cells (Astrup 1966). A quantitative method for assaying the activator in different tissues has been developed by Astrup & Albrechtsen (1957). They used potassium thiocyanate (KSCN) for extracting the tissue activator, which is firmly bound to structural proteins. The tissue activator obtained with this method is thermostable and acid stable (Astrup & Sterndorff 1956, Astrup 1966). Using this method it was found that the fibrinolytic activity of a given tissue varies with its vascularization (Albrechtsen 1957, Astrup 1966). High activities have been found in the human uterus, lymph nodes, adrenals, prostate and thyroid, and low activities in the testes, spleen and liver (Albrechtsen 1957). In cirrhotic liver, however, fibrinolytic activator activity is high (Todd 1959, Astrup et al 1960, Denk et al 1970).

The localisation of the fibrinolytic activity in different organs and tissues has been investigated also with the histochemical method of Todd (1959). In this method the amount of plasminogen activator is determined by covering sections of the tissue specimen with a thin film of fibrin and incubating for varying periods. In the histological sections lysed areas of the fibrin film are seen at the site of fibrinolytic activity (Kwaan & Astrup 1965, Pandolfi 1967, Bergstein & Michael 1972). Small amounts of plasminogen activator have been found in viable human neutrophilic granulocytes (Goldstein et al 1971, Wünschmann-Henderson et al 1972). In extensive studies of the vessels the fibrinolytic activity has been shown to be confined to the endothelium of the vasa vasorum in the adventitia (Todd 1959, Pandolfi 1969, Pandolfi et al 1969, Nilsson & Pandolfi 1970, Todd 1972). The endothelial cells of small vessels have also been suggested as the site of synthesis of the fibrinolytic activators (Chakrabarti et al 1963, Fearnley 1965, Pandolfi 1970, Nilsson & Pandolfi 1970). In tissue culture fibrinolytic activators are synthesised in explants of vessels, lungs, meninges, bone marrow and above all in explants of renal tissue (Painter 1961, Pandolfi 1970, Åstedt & Pandolfi 1972). The fibrino-

lytic activity in the kidney is localised mainly in the intralobular medulla and to the arcuate vessels and only to a minor extent in the renal cortex (Ladehoff 1960, Warren 1963, Kwaan & Fischer 1965, McConnel et al 1966, Pandolfi et al 1971, Åstedt & Pandolfi 1971).

Tissue activator has been purified from pig heart (Astrup 1951, Bachmann et al 1964) and from pregnant hog ovaries (Kok & Astrup 1969). The activator obtained from pregnant hog ovaries has been purified and found to have a molecular weight of 60,000. This activator was not identical with urokinase (Kucinski et al 1968, Thorsen & Astrup 1969, Kok & Astrup 1969).

Blood activator. Post-mortem blood has a high fibrinolytic activity, presumably because of increased permeability of the endothelium with a release of activating substances into the blood (Mole 1948, Müllertz 1952). The same explanation has been offered for the blood activator occurring in the circulation after other stimuli (Albrechtsen 1958, Kucinski et al 1968, Nilsson et al 1970, Nilsson & Pandolfi 1970). Blood activator, thus, most probably is derived from the vessel walls (Mole 1948, Kucinski et al 1968, Nilsson et al 1970). It has been suggested by Nilsson et al (1970) that the activator in the endothelium of small vessels is bound to other proteins or lipids and released in a labile form into the blood stream.

In extensive studies of the activation of plasminogen Müllertz demonstrated similarities in mode of action between the blood activator in post-mortem blood and the activity found after activation by streptokinase (Müllertz 1954a, 1956). Later Hamberg (1966) showed that the activator complex formed in normal plasma with streptokinase had a high molecular weight, while the activator isolated from post-mortem plasma had a molecular weight similar to that of albumin. The latter finding has been confirmed by Aoki & v.Kaulla (1971a), who found the molecular weight of an activator prepared from post-mortem blood to be 65,000. Auerswald et al (1971) also studied an activator prepared from post-mortem blood. The activator activity was in protein fractions with molecular weights of 30,500, 61,000 and 120,000. Most of the activity was found in the fraction with a molecular weight of 61,000 and it was suggested that the vascular plasminogen activator consists of polymers of a protein with a molecular weight of 30,500 or even half of that (15,000). Recently a

blood activator has been isolated from plasma. This activator had a molecular weight of 15-20,000 and behaved like tissue activator on chromatography on Sephadex G-100 (Hampton et al 1972).

Normally, only traces of activator occur in the plasma (Fearnley & Lackner 1955, Sherry et al 1959, Andersson et al 1962). In vivo, fibrinolytic activity is enhanced by various stimuli, such as physical exercise (Biggs et al 1947, Fearnley & Lackner 1955, Sherry et al 1959, Cash & Allan 1967, Prentice et al 1972, Britton et al 1973), electroshock (Müllertz 1957, Sherry et al 1959, Hilden & Lauritsen 1971) and operation (Macfarlane & Biggs 1948, Andersson et al 1962, Bennett et al 1967) as well as by other trauma (Bergentz & Nilsson 1961, Beard & Hampton 1963, Beard et al 1969). Increased fibrinolytic activity has also been demonstrated following the administration of various substances, such as epinephrine (Biggs et al 1947, Sherry et al 1959, Cash & Allan 1967, Cash et al 1970), nicotinic acid (Weiner et al 1958, Nilén & Nilsson 1964, Amery et al 1965, Robertson 1971), diguanides and certain anabolic steroids (Isacson & Nilsson 1970, Chakrabarti et al 1970, Davidson et al 1972). Venous occlusion of the limbs raises the fibrinolytic activity in the plasma (Clarke et al 1960, Robertson et al 1972). The enhancement of the fibrinolytic activity in response to all these types of stimuli is due to an increase in circulating labile blood activator (Sherry et al 1959, Nilsson et al 1970).

Urokinase is a β -globulin which occurs normally in the urine. It is an activator of plasminogen. Highly purified urokinase was prepared by Ploug & Kjeldgaard (1957), who used granulated silicic acid to adsorb urokinase from human urine. The purification procedure of Bergström (1963) includes adsorption of the urokinase activity to Hyflo Super-Cel at pH 5.0, elution with ammonium hydroxide and precipitation with ammonium sulphate. Purification procedures for urokinase were subsequently described by Celander & Guest (1960), Lesuk et al (1965) and White et al (1966). Its molecular weight has been found to be 53,000 (Lesuk et al 1965). Two active fractions have been found in human urine (White et al 1966, Schönebeck et al 1971). The molecular weights of these fractions were determined as 54,000 and 31,500, respectively (White et al 1966). Doleschel & Auerswald (1967) found not only these two similar fractions, but also a fraction of high molecular weight (104,000). Urokinase is stable within a

wide pH range (4-10) provided the temperature does not exceed 50°C (Ploug & Kjeldgaard 1957, Celander & Guest 1960, Nilsson et al 1970). Urokinase hydrolyses lysine and arginine esters (Alkjaersig et al 1958b, Celander & Guest 1960).

Urokinase is not identical with the blood activator (McConnell et al 1966, Kucinski et al 1968, Nilsson et al 1970, Aoki & v.Kaulla 1971a, Hampton et al 1972, Auerswald et al 1972) or with tissue activator (Kucinski et al 1968, Kok & Astrup 1969, Thorsen & Astrup 1969). Several studies indicate that urokinase is synthesised in the kidney and possibly also in the epithelium of the urinary tract (Ladehoff 1960, Painter & Charles 1962, McConnell et al 1966, Bernik & Kwaan 1967, Åstedt & Pandolfi 1971).

Streptokinase. In addition to the naturally occurring plasminogen activators already mentioned, human, but not bovine, plasminogen is activated by a bacterial product called streptokinase (Downie & Clifton 1950, Geiger 1952, Müllertz & Lassen 1953). Streptokinase is obtained from β -haemolytic streptococci as an α_2 -globulin with a molecular weight of 47,000 (Schwick & Heimbürger 1969, Heimbürger 1971). Müllertz & Lassen (1953) have shown that streptokinase reacts with a component in the euglobulin fraction (proactivator) to form an activator capable of converting bovine plasminogen to plasmin. They considered that a proactivator-activator system is involved in the activation of plasminogen (Müllertz & Lassen 1953, Müllertz 1954a, 1955, Troll & Sherry 1955). This proactivator interacted stoichiometrically with streptokinase (Müllertz 1955), forming an activator complex which was then assumed to convert plasminogen to plasmin. It has been shown that streptokinase reacts stoichiometrically with plasminogen with the formation of an activator complex (Ratnoff 1948, Wasserman 1952, Hummel et al 1966, Ling et al 1967, de Renzo et al 1967). In this complex an active site is generated before any proteolytic action on the plasminogen molecule is observed (McClintock & Bell 1971, Tomar & Taylor 1971, Reddy & Markus 1972), thus indicating the presence of two sites of the plasminogen molecule, one with activator activity and the other with proteolytic activity (Reddy & Markus 1972). It has, however, been demonstrated that plasmin too can form an activator complex with streptokinase, indicating that plasmin is another proactivator (Kline & Fishman 1961a, Ling et al 1965, Summaria et al 1971b, Reddy & Markus 1972). Streptokinase may, perhaps, also

activate plasminogen directly (Summaria et al 1969, Kline & Ts'ao 1971). It has recently been proposed (Taylor & Beisswenger 1973) that streptokinase interacts with plasmin to form modified streptokinase, which in turn acts on human plasminogen.

A specific globulin with proactivator activity has recently been isolated from the human euglobulin fraction (Takada et al 1970, 1971, 1972). It was a high molecular weight protein (mol.wt 300,000) and migrated electrophoretically together with α_2 -macroglobulin. These findings lend support to Müllertz' (1953a) theory that activation of plasminogen by streptokinase requires the presence of a particular globulin, the proactivator. The finding by Blix (1962) and Lauritsen (1969) that the level of proactivator activity but not that of plasminogen is lower in serum than in plasma also lends support to the theory of a specific proactivator protein or a change in the activator site of the plasminogen molecule during coagulation (Lauritsen 1969).

Inhibitors of the fibrinolysis fall into two groups, one inhibiting plasminogen activation and one acting like antiplasmin.

Evidence of the existence of a special inhibitor inhibiting the activation of plasminogen to plasmin was presented by Müllertz (1954a). The inhibition of the plasminogen activation has since been studied in systems containing urokinase (Nilsson et al 1961b, Paraskevas et al 1962, Brakman & Astrup 1963, McNicol et al 1963, Hawkey & Stafford 1964, Bennett 1967, 1970, Lauritsen 1968, Cucuianu et al 1972), streptokinase (Nilsson et al 1961b, Paraskevas et al 1962, Hawkey & Stafford 1964), tissue activator (Brakman et al 1966) and blood activator (Aoki & v.Kaulla 1971b). Some workers have found human serum to inhibit both urokinase and streptokinase (Nilsson et al 1961b, Paraskevas et al 1962, Lauritsen 1968), while others suggest the presence of an inhibitor acting specifically on the activation of plasminogen by urokinase (Brakman & Astrup 1963) or on the tissue activator (Brakman et al 1966, Thorsen 1973). Bennett (1967) suggested that the inhibitor of the urokinase activation of plasminogen inhibited also the blood activators.

Activator inhibitors with molecular weights of 43,000 (Kawano et al 1970) and 100,000 (Uszynski & Abildgaard 1971) have been prepared from placenta. Both inhibit

the activation of plasminogen by urokinase, but not that by streptokinase (Uszynski & Abildgaard 1971) or that by tissue activator from human heart (Kawano & Uemura 1971). In tissue culture placenta has been shown to inhibit urokinase as well as activators released from kidney and vessel explants (Åstedt et al 1972).

Little is known about the inhibitors of the plasminogen activation by urokinase in human serum. Gel filtration of human serum on Sephadex G-200 has, however, revealed two fractions with an inhibitory effect on human blood activator (Aoki & v.Kaulla 1971b). The molecular weight of the main fraction was 80,000 (Aoki & v.Kaulla 1971b).

An increased amount of inhibitors of the activation of plasminogen is important in the etiology of some cases of deep venous thrombosis (Nilsson et al 1961b, Brakman et al 1966).

Two types of antiplasmin have been demonstrated in plasma (Jacobsson 1955, Norman 1958, Norman & Hill 1958) viz the "immediately reacting" antiplasmin (α_2 -macroglobulin) and the "slow reacting" or "progressive" antiplasmin (α_1 -antiplasmin). The α_2 -macroglobulin has a molecular weight of 820,000, it is thermostable and reacts reversibly with plasmin (Ganrot 1966, Frenoy et al 1972). α_2 -macroglobulin inhibits not only plasmin, but also thrombin, trypsin, chymotrypsin, elastase and kallikrein (Mehl et al 1964, Ganrot 1966, 1967a, b, Steinbuch & Blatrix 1968, Steinbuch et al 1972, Rinderknecht & Geokas 1972).

It has been purified by Schultze et al (1963), Steinbuch et al (1965), Ganrot (1967b), Frenoy et al (1972), for example. Recently Frenoy et al (1972) showed that the molecule is most probably built up of 10 subunits each with a mol.wt of 83,000 and linked together by disulphide bonds. The molecule is a glycoprotein, its sedimentation constant is 19 S and its concentration in plasma has been estimated as 1.8 to 2.4 mg/ml. Its trypsin inactivating capacity is 0.06 mg/ml (Brown et al 1954, Schultze et al 1955, 1963, Weiss 1965, Ganrot & Scherstén 1967).

Kinetic studies have been performed by, among others, Ganrot (1967a,b) and Ganrot & Niléhn (1967), who found that 1 mole of α_2 M binds 1 mole of plasmin and 2 moles of trypsin. They also showed that plasmin and thrombin probably compete for the same site of the molecule. It was recently reported by Schreiber et al (1973) that one molecule of α_2 M inhibit two molecules of plasmin.

α_2 M has been found to be increased in children, pregnant women, women using oral contraceptives, patients with nephrosis or diabetes (Weiss 1965, James et al 1966, Ganrot & Bjerre 1967, Ganrot & Scherstén 1967), and decreased in patients receiving thrombolytic therapy with streptokinase or urokinase (Niléhn & Ganrot 1967, Arnesen & Fagerhol 1972).

The α_1 -antiplasmin is identical with α_1 -antitrypsin (Schultze et al 1962, Rimon et al 1966). It reacts as a progressive, irreversible antiplasmin (Shulman 1952, Jacobsson 1955, Norman 1958). It inhibits plasmin, thrombin, trypsin, chymotrypsin, elastase (Jacobsson 1955, Rimon et al 1966, Heimburger 1967, Gans & Tan 1967). The protein has been purified by Schultze et al (1955, 1962), Moll et al (1958), Bundy & Mehl (1959). Its molecular weight has been estimated as 47,000-60,000 (Schultze et al 1962, Rimon et al 1966).

The concentration of α_1 -antiplasmin in plasma is normally between 1.3 and 2.9 mg/ml (Störiko & Schwick 1964, Augener 1965). It is a glycoprotein, its sedimentation constant is 3.5 S and its trypsin inactivation capacity is 1.17 mg/ml (Moll et al 1958, Schultze et al 1962).

Its plasma level is raised in conditions with tissue damage and it reacts like an acute phase reactant (Kelley 1952, Shulman 1952). Thus, high levels have been seen in patients with cancer (Shulman 1952, Miesch et al 1971) and infections (Shulman 1952), in pregnant women (Faarvang & Lauritsen 1963, Ganrot & Bjerre 1967) and in patients with renal diseases, acute pancreatitis or diabetes (Miesch et al 1971). Low levels have been found in chronic obstructive lung disease (Laurell & Eriksson 1963, 1965, Eriksson 1965). An acquired α_1 -antiplasmin deficiency has been observed at least transiently in patients with massive liver necrosis (Campra et al 1973).

α_2 -macroglobulin and α_1 -antiplasmin vary independently of each other in certain diseases (Miesch et al 1971, Larsson et al 1971a,b) and during fibrinolytic therapy with streptokinase or urokinase (Niléhn & Ganrot 1967, Arnesen & Fagerhol 1972). According to Niléhn & Ganrot (1967) the α_2 -macroglobulin is presumably the most important antiplasmin because of its extreme fall in concentration during thrombolytic therapy with streptokinase, while the α_1 -antiplasmin remains unchanged. Recently Monkhouse et al (1972) found that the major part

of antiplasmin reacts immediately with the plasmin, neutralising but not destroying it.

In addition to the α_2 -macroglobulin and α_1 -antiplasmin, another plasma fraction called "inter- α "-inhibitor or protein π has been demonstrated (Steinbuch & Loeb 1961, Heide et al 1965, Heimburger & Haupt 1965, Steinbuch et al 1966). Its molecular weight has been given as about 180,000 (Steinbuch et al 1966) and it inhibits plasmin, and chymotrypsin (Steinbuch & Loeb 1961, Heide et al 1965). It was suggested by Ganrot (1967b) that this inhibitor is a complex between α_1 -antitrypsin and a proteolytic enzyme, although he failed to demonstrate that plasmin was part of the complex. The protein described by Steinbuch et al (1966) contains 1-1.2 moles of zinc/mole. This inhibitor has been purified by Heide et al (1965), Schwick et al (1966) and Steinbuch et al (1966). It is a glycoprotein with a sedimentation constant of 6.4 S. Its concentration in plasma has been found to be 3-5 % of the total amount of inhibitor (0.1-0.3 mg/ml plasma) and to have a trypsin inactivating capacity of 0.04 mg/ml (Heide et al 1965, Heimburger & Haupt 1965, Schwick et al 1966).

The antiplasmins have also been characterised and separated by utilisation of differences in their reaction to heating to 56°C for 30 minutes, by treatment with 0.25 M methylamine and by acidification (pH 2.0) (Shulman 1952, Norman & Hill 1958, Mehl et al 1964, Rimon et al 1966, Schwick et al 1966, Rowley & Oette 1973). The α_2 M and the inter- α -inhibitor are thermostable, but not α_1 -antiplasmin. Treatment with methylamine inactivates the α_2 M, but not the other inhibitors. Acidification to pH 2.0 virtually destroys both α_2 M and the α_1 -antiplasmin activities while the inter- α -inhibitor is acid-stable.

Immunochemical studies of these three antiplasmins have shown that they are not identical (Ganrot & Bjerre 1967, Miesch et al 1971).

It has been recently shown by Müllertz (1972) that two molecular forms of plasmin develop in post-mortem plasma, one of which associates with α_2 -macroglobulin with a relatively high association constant, the other probably forming a complex with an so far unknown inhibitor. This inhibitor was shown not to be α_1 -antiplasmin or inter- α -inhibitor. These findings thus suggest that α_1 -antiplasmin as well as inter- α -inhibitor is of little significance in the activation of the fibrinolytic system post-mortem.

Synthetic inhibitors. As early as 1954 it was shown that aliphatic amino acids, such as lysine and ornithine and esters of lysine and arginine, inhibit the fibrinolytic system (Müllertz 1954, Troll et al 1954). The esters of lysine and arginine were shown to be competitive inhibitors of plasmin (Troll et al 1954) as well as of the activator activity of urokinase and streptokinase (Alkjaersig et al 1958b). Sjoerdsma & Nilsson (1960) found that several aliphatic amino acids have an inhibitory effect on the activation of plasminogen by streptokinase. The strongest effect was obtained by the amino acids with a carbon chain length of 4-8 and with the amino group in the omega position. Synthetic amino acids, such as E-aminocaproic acid (EACA) (Okamoto et al 1957, Ablondi et al 1959, Nilsson et al 1960), tranexamic acid (4-aminomethyl cyclohexane carboxylic acid, AMCA) (Okamoto & Okamoto 1962, Andersson et al 1965, 1971) and aminomethyl benzoic acid (PAMBA) (Markwardt et al 1967) have been found to be strong inhibitors predominantly of the activation of plasminogen. They act as competitive inhibitors of the activation of plasminogen and in higher concentrations as non-competitive inhibitors of plasmin (Alkjaersig et al 1959a, Johnson et al 1969b). It has since been demonstrated that both EACA and AMCA form a stoichiometric complex with plasminogen. This results in changes in the plasminogen molecule with a consequent change in sedimentation constant of the plasminogen and an increase in the molecular weight (Alkjaersig et al 1959a, Abiko et al 1969, Brockway & Castellino 1971). The changed plasminogen molecule has no ability to react with streptokinase and other activators and then acts like a competitive inhibitor of the activation of plasminogen by streptokinase and urokinase (Johnson et al 1969b, Brockway & Castellino 1972). It has recently been suggested that the antifibrinolytic activity of free amino acids is due to conformational alteration in the plasminogen molecule induced by these compounds (Brockway & Castellino 1972).

Tissue bound inhibitors. Inhibitors of fibrinolysis have been found in various organs such as lung, pancreas and the seminal vesicles (Kunitz & Northrop 1936, Astrup 1950, Fritz et al 1968). Pugatch et al (1970) found inhibitory activity in material obtained from bovine mesothelium lining the parietal peritoneum of the abdomen and visceral pleura of the lungs. An inhibitor has been isolated from bovine pancreas, Trasylol, which inhibits plasmin as well as the activation of

plasminogen (for ref. see Ann N Y Acad Sci 146, Art 2 1968). This inhibitor has been used clinically (Andersson et al 1967, Matis & Mörl 1968, Phillips et al 1968). Human platelets contain inhibitors of fibrinolysis with both an antiplasmin and with an inhibitory effect on the activation of plasminogen (Johnson & Schneider 1953, den Ottolander et al 1969a,b, Ganguly 1972). The antiplasmin effect is weak (Ekert et al 1970), while the antiactivator effect is strong, especially on urokinase (Kwaan & Suwanwela 1971, Ogston et al 1973). An activator from human platelets has been partially purified by chromatography on DEAE-cellulose by Ogston et al (1973). This inhibitor inhibited both urokinase and tissue activator prepared from human heart. The molecular weight was found to be about 45,000 and it was destroyed by heating to 56°C for 30 minutes. It was supposed to be released on the platelet aggregation. It has recently also been found by others that the antiplasmins of the platelets are released after addition of, for example, collagen or ADP to the platelets (Ulutin & Aktuglu 1973, Dechavanne et al 1973).

Extracts of cells from liver carcinoma possess anti-fibrinolytic activity, which has been ascribed predominantly to an inhibitory effect of the activation of plasminogen (Kwaan et al 1959). Using a modified fibrin slide technique Noordhoek Hegt & Brakman (1973) have demonstrated inhibitors of fibrinolysis in human tissue, such as liver, pancreas and vascular tissue.

Fibrinogen is a macromolecule with a molecular weight of 340,000 (Blombäck & Yamashina 1958, McDonagh et al 1972, Blombäck 1972). It contains three peptide chains, A α (mol.wt 64,000), B β (mol.wt 57,000) and γ (mol.wt 48,000) (Blombäck & Yamashina 1958, Gaffney & Dobos 1971, McDonagh et al 1972). The carbohydrate content of the fibrinogen molecule has been shown to be localised to the B β - and γ -chains, while the A α -chains do not contain any carbohydrate (Gaffney 1972a). The chains are linked together by disulphide bonds. 40 % of the disulphide bridges are located in the hydrophilic N-terminal portion in the so-called disulphide knot (mol.wt 60,000) (Blombäck et al 1968, Blombäck 1972). Three additional disulphide knots, which are hydrophobic, have recently been demonstrated by Gårdlund et al (1972). The molecular weight of one of the hydrophobic disulphide knots is about 30,000 and it contains 4-6 disulphide bridges. Two polypeptide chains are included, one which has been shown to be B β (Gårdlund et al 1972). Recently Timpl & Gollwitzer (1973) found three

disulphide-containing peptides after CNBr-treatment of the fibrinogen molecule. The largest peptide they assumed to correspond to the N-terminal disulphide knot described by Blombäck et al (1968).

Fibrinogen occurs in plasma in a concentration of 0.29 ± 0.07 g/100 ml (Collen et al 1972b). Plasma fibrinogen is heterogeneous, as judged from its solubility (Blombäck & Blombäck 1956, Mosesson & Sherry 1966, Finlayson et al 1972). The readily soluble material has a lower molecular weight (Mosesson et al 1967) and consists of molecules with partly degraded A α -chains (Mills & Karparkin 1970, Mosesson et al 1972a). These authors found that the N-terminal portion of the chains are intact and that cleavage of any or all of at least 10 sites along the A α -chain results in the formation of early catabolic intermediates. Another type of heterogeneity of the fibrinogen resulting in a charge difference depends on the presence of a γ -chain with a greater anodal mobility at both pH 8.6 and pH 2.7 on gel electrophoresis. In addition, two types of γ -chains have been found which on gel electrophoresis differ in their behaviour only at pH 2.7. These slightly modified γ -chains have, however, a normal ability to take part in the transamidation reaction, thus indicating that their C-terminal regions are intact and are of the same molecular size (Gerbeck et al 1969, Chen & Doolittle 1971, Mosesson et al 1972b). Recently Henschen & Edman (1972) found three types of γ -chains after chromatography on CM-cellulose of S-carboxymethyl fibrin. The two minor fragments differed from the main fraction in their amino acid composition.

The N-terminal part of the molecule contains the fibrinopeptides A and B, which are located in the A α - and B β -chains, respectively and are split off by the action of thrombin on the fibrinogen molecule (Bailey et al 1951, Lorand 1951, Bailey & Bettelheim 1955). Thrombin splits arginyl-glycine bonds in the A α - and B β -chains (Blombäck 1971, Blombäck et al 1972) and releases N-terminal residues of glycine in the fibrinogen molecule (Bailey et al 1951, Blombäck & Yamashina 1958, Kierulf & Abildgaard 1971). The fibrin monomers aggregate, first end-to-end and then side-to-side. Under the influence of factor XIII (fibrin stabilising factor, FSF) and Ca²⁺ the fibrin clot stabilises and becomes insoluble in 8 M urea och 1 % monochloroacetic acid. This so-called cross-linking is a transamidase catalysed reaction with the formation of covalent bonds between the glutamyl residue in one fibrinogen mole-

cule and the amine group of the lysine residue of another fibrinogen molecule (Lorand et al 1968, Lorand 1970, McKee et al 1970, Chen & Doolittle 1971).

Fibrinogen is believed to be synthesised in the liver (Forman & Barnhart 1964, Adelson 1968, Miller & John 1970). Also extrahepatic fibrinogen formation has been assumed since the plasma fibrinogen level is often normal in various types of liver damage (Hallén & Nilsson 1964, Donaldson et al 1969, Ansari & Silvis 1972). Collen et al (1972b) found the half-life of fibrinogen to be 4.14 ± 0.56 days. They also reported that neither heparin treatment nor treatment with tranexamic acid influenced the fibrinogen turnover in normals. They concluded that in health neither intravascular fibrin formation nor fibrinogenolysis is essential in the turnover of fibrinogen (Collen et al 1972b). In contrast, Cultrera et al (1972) found a prolongation of the half-life time after administration of tranexamic acid.

Fibrin stabilising factor (FSF, factor XIII). The formation of a stabilised fibrin clot occurs by transamidation under action of activated factor XIII, which is a transamidase. This occurs by formation of covalent bindings between a glutamyl residue on one fibrin monomer and a lysine residue on the other.

Two such cross-links are formed between two γ -chains of the fibrinogen, and four others between α -chains, but the β -chains are not cross-linked. Thus 6 moles of glutamyl-lysine cross-linkings are formed per mole of fibrin (Pisano et al 1968, 1972, McKee et al 1970, Ball et al 1972).

It has been shown that the cross-linking site of the γ -chain resides in the D-fragment obtained on plasmin digestion of fibrinogen (Jamieson & Gaffney 1968, Pizzo et al 1972, Gaffney 1972b, Gaffney & Brasher 1973).

Factor XIII occurs in plasma (Laki & Lorand 1948, Lorand & Konishi 1964), in platelets (Lüscher 1957, McDonagh et al 1969b, McDonagh & McDonagh 1972, Schwartz et al 1973) and in placenta (Bohn & Schwick 1971). The inactive precursor protein, FSF, is converted to an active transpeptidase called fibrinolygase (FSF*) by the action of thrombin (Lorand & Konishi

1964, Lorand et al 1968, Tyler 1970). Trypsin, papain, and reptilase have also been found to activate FSF (Lorand et al 1968, Konishi & Takagi 1969). Calcium ions are known to accelerate the aggregation of soluble fibrin monomers or polymers (Ratnoff & Potts 1954, Lorand & Konishi 1964, Boyer et al 1972). The molecular weight of FSF is 320,000 and the molecule is believed to include two types of polypeptide chains (a and b). Each molecule consists of two a-chains and two b-chains. The a-chain of the platelet-FSF has been shown to be identical with the a-chain of plasma-FSF. The platelet-FSF has a molecular weight of 146,000 and consists of two a-chains (McDonagh & Wagner 1970, Schwartz et al 1971, 1973, Takagi & Konishi 1972). According to Bohn et al (1972, 1973) the plasma-FSF is composed of two subunits of A-type (molecular weight 80,000) and one inactive subunit called S (molecular weight 180,000). The subunits are noncovalently combined. The platelet- and placenta-FSF consists of two noncovalently coupled A-subunits. McDonagh & McDonagh (1972) found that the platelets contain one sort of platelet transamidase, which requires thrombin activation and was identified as platelet factor XIII. They also found a transamidase, which did not require thrombin activation. This enzyme did not seem to be related to platelet factor XIII. During platelet aggregation with ADP, factor XIII was released from the platelets.

Lack of FSF has sometimes been found in families with a hereditary haemorrhagic diathesis (Duckert et al 1960, Ikkala & Nevanlinna 1962, Lorand et al 1970, Stefanini et al 1972). Low levels of FSF have been found predominantly in liver and renal diseases (Nussbaum & Morse 1964, Walls & Losowsky 1969, Losowski & Walls 1971) as well as in newborn infants (Künzer & Lütgemeier 1965, Ambrus et al 1970, Henriksson et al 1973). Inhibitors of FSF have been demonstrated to lead to haemorrhagic syndromes (Lewis et al 1967, Lorand et al 1972).

Fibrin/fibrinogen degradation products (FDP). The breakdown of plasmin results in the formation of various types of degradation products: first the high molecular weight FDP (HMWDP), designated X- (mol.wt 240,000) and Y-fragments (mol.wt 155,000) by Marder et al (1967) and Marder & Shulman (1969a). The X-product is still coagulable with thrombin, while the Y-product is not. This indicated that the plasmin digestion of the A α -chains occurs from the COOH-terminal parts of the chains. Mosesson et al (1967) also showed that the NH₂-terminal groups of fibrinogen are unchanged during the initial stages of plasmin action. The A α -chain of the fibrinogen molecule does not contain any carbohydrate and has been shown to be most susceptible to plasmin degradation (Pizzo et al 1972, Gaffney 1972a), while the γ -chain on the last one to be degraded by plasmin (Pizzo et al 1970, Mills & Karparkin 1970, Gaffney & Dobos 1971, Mills 1972). Fragment X has been found to be a heterogeneous population with different molecular weights (248,000-252,000) because of different degrees of degradation of A α - and B β -chains (Pizzo et al 1972). The appearance of fragment X is accompanied by the appearance of A-, B- and C-peptides (Nussenzweig et al 1961a, Marder & Shulman 1969a). These are peptides split off the A α -chain and do not possess the antigen properties of fibrinogen (Pizzo et al 1972). When fragment Y (mol.wt 150,000) is formed, a D-fragment is also split off (Marder et al 1967, Pizzo et al 1972, Furlan & Beck 1972). Fragment Y contains the disulphide knot of the fibrinogen molecule and partially degraded A α -, B β - and γ -chains, one of each. Further degradation of the Y-fragment results in the formation of a D- and a E-fragment. The D- and E-products, often called the end degradation products, have antigenic determinants in common with fibrinogen (Nussenzweig et al 1961b, Marder et al 1967). It has been shown, first, that the molecular weight of the D-fragments varies between 83,000 and 100,000 according to the extent of degradation of the γ -chain, second, that the D-fragment derived from cross-linked fibrin differs from that derived from fibrinogen in that it contains a dimer of the degraded γ -chain including the cross-linking site of the molecule and two each of the degraded α - and β -chains, third, that the molecular weight of the E-fragment is 41,000 and, fourth, that the E-fragment consists of the disulphide knot of the fibrinogen molecule (Wallén 1971, Pizzo et al 1972,

minants of fibrinogen (Merskey et al 1971).

FDP have been shown to have anticoagulant effects by acting as antithrombin or as inhibitors of the polymerisation of the fibrin monomers with prolongation of the thrombin time as a result. It has, however, been shown that only FDP concentrations of more than 500 $\mu\text{g/ml}$ markedly prolong the thrombin time and thus question the clinical significance of the effect (Niléhn 1967b, Hedner & Nilsson 1972, Arneson & Godal 1973). The D-fragment acts as an inhibitor of fibrin polymerisation (Alkjaersig et al 1962, Latallo et al 1962, Catanzaro et al 1971, Larrieu et al 1972, Arneson & Godal 1973), while the E-fragment has an antithrombin effect (Larrieu et al 1972, Arneson & Godal 1973). The high molecular weight FDP, especially the X-products, act as inhibitors of fibrin polymerisation (Marder & Shulman 1969b, Latallo 1972, Arneson & Godal 1973).

At least 50 % of the high molecular weight degradation products are retained in the fibrin clots (Niléhn 1967a, Larrieu 1971). The in vivo behaviour of human fragment D in rabbits has been studied by Hayne & Sherman (1973). They found two components in plasma clearance of I^{125} fragment D viz 66.0 ± 6.0 % was cleared with a $T/2$ of 0.9 ± 0.2 hrs and 32.3 ± 5.3 % with a $T/2$ of 3.6 ± 0.3 hrs. They found the same clearance in hepatectomised and RES-blockaded rabbits while nephrectomised animals showed a normal first component while the second one was prolonged ($T/2$ 6.0 ± 1.0 hrs). The authors concluded that the kidneys are important in the catabolism of one portion of fragment D.

Niléhn (1967b) found FDP to have no effect on platelet adhesiveness in vitro, while Kowalski (1968) reported such an effect of both "early" and "late" FDP. It has been claimed that the FDP derived from fibrinogen inhibit platelet aggregation (Kopec et al 1966, Latallo 1972), while those derived from fibrin stimulate platelet aggregation (Latallo 1972). Platelet aggregation is inhibited by the low molecular weight dialysable fragments split off during plasmin digestion of fibrinogen (Larrieu 1971). Recently it has also been shown that peptides produced by streptokinase induced fibrinogenolysis as well as peptides obtained during digestion of serum by trypsin exerted an inhibitory effect on the platelet aggregation thus indicating an unspecific anti-aggregating activity of degradation products (Solum et al 1973). The same authors found that low molecular

weight degradation products showed significant aggregation inhibition only in high concentrations.

A kinin-like effect, including vasoconstriction, increase in blood pressure and in capillary permeability, has been attributed to the low molecular peptides (A-, B-, C-peptides) split off during digestion of fibrinogen by plasmin (Malofiejew 1971).

METHODS

Collection of blood. The blood was collected into siliconised centrifuge tubes containing 1 volume of 3.8 % trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \times 2 \text{ H}_2\text{O}$) solution. The blood samples were immediately centrifuged at 2,000 g for 20 minutes. The plasma was withdrawn by means of siliconised pipettes and distributed among plastic tubes. The plasmas were stored at -70°C until used.

For preparation of serum the blood was collected in glass tubes. After standing 2 hours at room temperature the tubes were centrifuged at 2,000 g for 20 minutes. The serum was then carefully withdrawn by means of siliconised pipettes and dispensed in glass tubes (2 ml in each). The serum samples were immediately stored at -20°C . Each sample was used only once.

Collection of urine. Morning urine (I) or a 24 hour volume (II, III) were collected without addition of EACA. The samples were stored frozen until tested. Concentration of the 24 hour volume was performed by ultrafiltration with a membrane that retained proteins with a molecular weight of more than 12,000 (Diaflo Ultrafilter Membrane, Amicon Corporation, Massachusetts) and by dialysing against polyethylene glycol until the protein concentration was about 70 g/l (Biuret method).

Platelet count (Björkman 1959). Normal range 155,000-365,000/ mm^3 .

Platelet adhesiveness. The whole blood of Hellem (1960) as modified by Cronberg (1968). Normal range 17-35 %.

Coagulation time in (a) glass tubes (Nilsson et al 1961a), normal range 6-14 min, and (b) plastic tubes (Cronberg 1968), normal range 15-25 min.

Recalcification time of plasma (Nilsson et al 1961a). Normal range 120-160 sec.

One-stage prothrombin time (Owren 1953). Normal range 15-17 sec.

Prothrombin + factor VII + factor X (Owren's P&P-test; Owren & Aas 1951). Normal range 80-120 %.

Factor V (Wolf 1953). Normal range 80-120 %.

Thrombin time (Nilēhn & Nilsson 1964). Normal range 16-24 sec.

Fibrinogen (Nilsson & Olow 1962a). Blood was collected with E-aminocaproic acid (EACA). Normal range 0.20-0.40 g/100 ml.

Euglobulin clot lysis time (Nilsson & Olow 1962b). Normal >100 min.

Spontaneous fibrinolytic activity of plasma and resuspended euglobulin precipitate on unheated and heated fibrin plates (Nilsson & Olow 1962b). Normal range for plasma 0-50 mm²; resuspended euglobulin precipitate 0-70 mm².

Fibrinolytic response to venous stasis of the arms (Robertson et al 1972). The fibrinolytic activity of resuspended euglobulin precipitate on unheated fibrin plates was determined after 20 minutes of venous occlusion. Normal range for resuspended euglobulin precipitate >157 mm².

Fibrinolytic activity in the vein vessel wall (Pandolfi 1972). Venous biopsy specimens obtained from the dorsal side of the hand were quickly frozen. Cryostat sections of the specimens were covered with a plasminogen-rich fibrin layer on glass slides. After incubation at 37°C for various times the fibrinolytic activity was measured as the lytic areas formed in the fibrin film. Normal range 6.0-10.0 arbitrary units.

Plasminogen (1) clot method (Nilsson et al 1957b, Hedner et al 1970, V). Normal range 60-140 %; (2) caseinolytic method (Hedner & Nilsson 1965). Normal range 8-19 ACU/ml. 10 ACU = 1 CTA unit; (3) immunochemical method (Ganrot & Nilēhn 1968) as modified by Ekelund et al (1970). Blood collected with EACA. Normal range 70-130 %.

Fibrin(ogen) degradation products (FDP). Blood samples were collected in tubes containing EACA to prevent in vitro fibrinolysis. Serum was prepared according to Paraskevas et al (1962). No EACA was added to the urine. FDP in both serum and urine were determined according to the immunochemical method of Nilēhn (1967a) using a specific antiserum against the D-product in the rocket method of Laurell (1966). The serum (dil 1/1) and urine samples (dil 1/1, both unconcentrated and concentrated urine) were applied to agarose plates (1 % agarose)

containing the anti-D-serum and on high voltage electrophoresis (20 V/cm) the antigen was forced into the gel. Precipitation peaks form, and their heights are proportional to the amount of antigen in the sample. High molecular weight degradation products (HMWDP) were used as standard. With this no FDP could be demonstrated in serum or urine from 200 apparently healthy controls (Hedner & Nilsson 1971). Neither were any FDP found in concentrated 24 hour urine volumes from 19 controls (II).

Typing of FDP was performed according to Bouma et al (1971; I).

Preparations of fibrinogen degradation products (FDP). 100 ml of a fibrinogen solution (human fibrinogen, 10 g/l, AB Kabi, Stockholm, Sweden) was degraded with 35 CTA units of plasminogen (glycerol activated plasmin, AB Kabi, Stockholm, Sweden) at room temperature. High molecular weight degradation products (HMWDP, X- and Y-fragments) were prepared essentially according to Marder & Shulman (1969) with a digestion time of 10 minutes followed by gel filtration on Sephadex G-200 (AB Pharmacia, Uppsala, Sweden; phosphate buffer, 0.01 M, pH 7.8). The end products, D- and E-products, were prepared essentially according to Nilēhn (1967a). When preparing the E-product the fibrinogen digest was heated (56°C for 30 min) to denaturate the D-product before chromatography, which was performed on DEAE-cellulose (Serva Entwicklungslaboratorium, Heidelberg, Germany) in the same way as for the D-product (2.5 x 45 cm column; phosphate buffer, 0.01 M, pH 8.6; eluted with 0.3 M potassium dihydrogen phosphate solution; elution rate 96 ml/hour). The various solutions with degradation products were concentrated by dialysis in 23/32 Visking dialysis tubes against polyethylene glycol (Carbowax 20, 300 g/l).

Inhibitors of plasminogen activation were determined according to (a) a clot lysis method described by Paraskevas et al (1962). Inhibition of the urokinase activation: in this system the following substances were added to each tube in the order given: 0.5 ml of the serum sample (dil 1/5, 1/10, 1/20) or the activator inhibitor fraction in 0.9 % NaCl + 0.1 ml plasminogen (0.01 mg/ml in 0.9 % NaCl) + 0.2 ml bovine fibrinogen (0.5 % in Tris buffer, pH 7.8, 0.07 M, ionic strength 0.15) + 0.1 ml urokinase (200 Ploug units/ml in aq. dest.) + 0.1 ml thrombin (100 N.I.H.units/ml in 0.9 % NaCl). The inhibition of the streptokinase activation

was determined as follows: 0.5 ml of the substances to be tested in 0.9 % NaCl + 0.2 ml human fibrinogen (0.5 % in Tris buffer, pH 7.8, 0.07 M, ionic strength 0.15) + 0.1 ml streptokinase (Kabikinase^R, 50 I.U./ml in 0.9 % NaCl) + 0.2 ml thrombin (100 N.I.H. units/ml in 0.9 % NaCl). The lysis time was calculated from the moment of addition of urokinase or streptokinase. The inhibitory effect of the samples was expressed relative to a standard consisting of pooled serum from 25 apparently healthy persons. Two blanks were used in which 0.5 ml 0.9 % NaCl and 0.5 ml of 0.1 % EACA-solution, respectively, were added. The EACA was used to check the results of tests performed on different occasions. Normal range for the urokinase inhibitors: 60-140 %; SD $\pm$ 9 %; (b) a caseinolytic method first described by Lauritsen (1968) and modified by Hedner et al (1970; V). To 0.5 ml serum (dil 1/1) were 0.5 ml of urokinase (200 Ploug units/ml) and 0.5 ml of porcine plasminogen (0.3 CTA units/ml) added. After an activation time of 40 min at 37°C, 0.5 ml 2 M EACA was added. 2 ml casein (3 % prepared according to Müllertz 1955) was added after incubation for 20 minutes at 37°C and after 60 minutes of caseinolysis, 1 ml perchloric acid (2 N). The absorbance was read in a Beckman DU apparatus at 280 nm. The value obtained is a measure of the total inhibitor effect i.e. antiplasmin + the inhibition of the activation (B-value). To the sample used only for measuring the antiplasmin activity, the test samples were added after EACA (A-value). The difference between the absorbance of the sample used to measuring the total inhibitory effect and that used for measuring only the antiplasmin effect is an estimate of the inhibitors of plasminogen activation.

α 2-macroglobulin. (1) Esterolytic method (Ganrot 1966). Normal range in serum 80-120 %; (2) immunochemical method with the rocket method of Laurell (1966). Normal range in serum 60-170 %.

Antiplasmin. (1) Caseinolytic method by Shamash & Rimon (1964), as modified by Ekelund et al (1970). Normal range 400-600 ACU/ml. (2) Fibrin plate method (Nilsson et al 1961b). Normal range 60-140 %.

Total antitrypsin activity (TAT). Esterolytic method (Eriksson 1965). Normal range 0.7-1.4 mg/ml.

Antithrombin III, ceruloplasmin, haptoglobin, albumin and lysozyme. Immunochemical methods using the rocket technique of Laurell (1966). Normal ranges: ceruloplas-

min 23-44 mg/100 ml, haptoglobin 30-170 mg/100 ml, antithrombin III 75-122 %, albumin in urine <50 mg/l, lysozyme <3 mg/l (Johansson & Malmquist 1971).

Urokinase activity of the urine was determined in unconcentrated urine with Anderson's clot method (1962). The urokinase in diluted urine (1/5) activated added plasminogen and the plasmin formed was allowed to lyse a fibrin clot made from bovine fibrinogen and thrombin. The time for complete lysis was taken as a measure of the urokinase activity. In 10 control persons values above 8 Ploug units/ml were obtained (III).

Creatinine concentration in serum and urine as well as blood urea nitrogen (BUN) was measured by a Technicon Autoanalyser and creatinine clearance determined at the Department of Clinical Chemistry.

Protein concentration according to the method of Kjeldahl.

Gel filtration on Sephadex G-200 (AB Pharmacia, Sweden). 5 ml of serum or concentrated urine was applied on a 2.5 x 45 cm column at +4°C. The column was equilibrated with 0.07 M Tris-HCl buffer (pH 7.8, ionic strength 0.15) and was eluted at a rate of 10 ml/hour. The effluent was collected in 3 ml portions and the absorbances of the fractions were measured at 280 nm in a Beckman DU spectrophotometer.

Ion-exchange chromatography on DEAE-Sephadex (AB Pharmacia, Sweden) was performed at +4°C on a 1.5 x 30 cm column. The column was equilibrated with 0.01 M phosphate buffer (pH 8.6) and was eluted with the same buffer (VIII). The elution rate was 10 ml/hour. The effluent was collected in 3 ml portions. The absorbances of the fractions were measured at 280 nm in a Beckman DU spectrophotometer. All the fractions were dialysed against 0.07 M Tris-HCl buffer (pH 7.8, ionic strength 0.15) before the different inhibitors were assayed.

Analytical agarose electrophoresis of urine (II) or the inhibitor fraction (VIII) was performed in 1 % agarose (1 mm thick) in a barbital buffer (0.075 M, pH 8.6 containing calcium lactate) and run for 3 hours. The gel was coloured with amide black. Pooled normal serum served as reference.

Preparative gel electrophoresis was performed (VIII) in 1 % agarose gel, 2 mm thick. The samples, to which 3 % agarose solution had been added, were applied in troughs (5 x 80 mm). The electrophoresis was run at 200 V for about 1/2 hour in a barbital buffer (0.075 M; pH 8.6 containing calcium lactate). A normal serum with an albumin marker was run in the same gel. After the electrophoresis the gel was cut into 0.5 cm slices, which were placed in tubes and frozen at -70°C for 30 minutes. They were afterwards thawed at room temperature and centrifuged (at 2,000 g) for 30 minutes. The supernatant was removed and tested.

Analytical polyacrylamide gel electrophoresis according to Davis (1964).

Covalent coupling of haemoglobin to activated agarose was performed with the method of Axén et al (1967). The haemoglobin was activated with cyanogen bromide in alkaline environment and then coupled to the gel in alkaline aqueous medium.

Molecular weight was determined on Sephadex G-100 according to the method of Whitaker (1963). Reference substances: gammaglobulin (mol.wt 160,000), trypsin (mol.wt 23,800), pepsin (mol.wt 35,000) and albumin (mol.wt 70,000). Five determinations were made of each substance.

Immunological identification of the different antibodies obtained in the antiserum raised in rabbits against the partially purified inhibitor fraction (VIII) was performed according to Kröll (1968).

A. Fibrin/fibrinogen degradation products in renal diseases

Fibrin deposits in the kidney. With immunofluorescent techniques and electron microscopy several workers have demonstrated intraglomerular accumulation of fibrin/fibrinogen and of substances immunologically related to them in experimental and various types of human glomerulonephritis; in the nephritis of SLE; and in eclampsia (Paronetto & Strauss 1962, Simon & Chatelanat 1963, Vassalli et al 1963, Morris et al 1964, Paronetto & Koffler 1964, Vassalli & McCluskey 1965, Koffler & Paronetto 1965, 1966, Paronetto 1965, Bacani et al 1968, Humair et al 1969, Salmon & Lambert 1971, Clarkson et al 1971, Chirawong et al 1971, Churg & Grishman 1972, Davison et al 1973). Such deposits occur also in acute renal graft rejection and in the haemolytic-uraemic syndrome (McKay & Margaretten 1967, Starzl et al 1968, Habib et al 1969, Luke et al 1970, Franklin et al 1972, Salaman 1972, Lieberman 1972, Claes et al 1973).

It has been assumed that the fibrin deposits are the result of a disseminated intravascular coagulation precipitated by the primary renal disease (McKay 1965, Hardaway 1966, Wardle & Taylor 1968, Haanen et al 1971a). However, most renal diseases are characterised by a normal platelet count, high levels of AHF and fibrinogen, high or normal levels of factor V, prothrombin and plasminogen, low fibrinolytic activity, and absence of fibrin monomers in the circulating blood even when FDP are demonstrable in the serum and/or, above all, in the urine (Wardle & Taylor 1968, Larsson et al 1971c). It has been questioned whether disseminated intravascular coagulation is necessary for the formation of fibrin deposits in the kidney and suggested that the deposits are caused by local coagulation (Rodriguez-Erdmann & Guttman 1969, Gilchrist & Lieberman 1969, Nilsson 1970, Merskey 1970, Katz et al 1971, Kleinknecht & Kaufer 1973). Merskey (1971) stressed that fibrin deposits in the kidney may be caused by the action of enzymes released from damaged cells, not necessarily by activation of the blood clotting system.

In 1968 Katz et al showed that infusion of antibodies to connective tissue results in fixation of the antibodies to the glomerular capillary walls and basement

membranes in the glomeruli. Two immunopathological causal mechanisms have been suggested for glomerulonephritis: 1) antibodies react with circulating antigens to form nonglomerular antigen-antibody complexes which can fix complement and be deposited along the basement membrane; and 2) antibodies to a specific antigen present in the basement membrane react with the basement membrane and fix complement (Wilson 1972, Mauer et al 1973, McCluskey & Klassen 1973). Local cell injury may then be caused by enzymes released from the leukocytes which accumulate on complement fixed to the antigen-antibody complexes. This endothelial injury leads to exposure of the basement membrane to the blood-stream. The collagen of the basement membrane may induce platelet aggregation, and this in turn may lead to development of fibrin thrombi (Franklin et al 1972).

Digestion of fibrin/fibrinogen requires at least local fibrinolytic capacity in the kidneys.

Fibrinolytic activators in the kidney. Two types of fibrinolytic activators occur in the kidney viz tissue plasminogen activator and urokinase. With the histochemical method of Todd (1959) the tissue activator is found to be localised mainly to blood vessels in the medulla and to the large intrarenal blood vessels (Warren 1963, Kwaan & Fischer 1965, McConnell et al 1966, Pandolfi et al 1971). Pandolfi et al (1971) found that renal cortex acquires fibrinolytic properties in chronic inflammation and during rejection of renal grafts, while normal human glomeruli were inactive. In foetal kidneys Åstedt & Pandolfi (1971) found activity histochemically also in the intralobular arteries and veins but not in the glomeruli. Others have found fibrinolytic activity in the glomeruli of normal kidneys (Epstein et al 1968, Bergstein & Michael 1972) in the presence as well as in the absence of intraglomerular fibrin, but not in renal grafts undergoing progressive rejection (Bergstein & Michael 1972). Bernik & Kwaan (1967) found fibrinolytic activity in cultures of human renal explants which they thought was due to both tissue fibrinolytic activator and urokinase released from the explants. Åstedt & Pandolfi (1972) found both cortex and medulla in organ culture of normal foetal kidneys to be extremely fibrinolytically active although no lytic activity was demonstrable histochemically in the glomeruli. They suggested that the fibrinolytic activity of

living renal tissue is probably due mainly to the production of urokinase. An activator of plasminogen identical with urokinase is produced by kidney cell cultures (Painter & Charles 1962, Kucinski et al 1968, Bernik & Kwaan 1969).

The fibrinolytic system in the circulating blood in renal diseases. Systemic fibrinolytic activity in renal disease is extremely low both in the blood (Edward et al 1964, McNicol et al 1965, Sharma et al 1967, Hutton & O'Shea 1968, Wardle & Taylor 1968, Wardle et al 1970, Larsson et al 1971a, Ito et al 1972) and in most tissues (MacLeod et al 1962). In uraemia components of the fibrinolytic systems have been much investigated. Plasminogen concentration has been found to be normal, while the fibrinogen has proved to be increased in most studies (Edward et al 1964, McNicol et al 1965, Wardle & Taylor 1968, Larsson et al 1971a, Ekert et al 1972b). The low level of spontaneous fibrinolytic activity may be due to decreased activity of fibrinolytic activators or increased amounts of the fibrinolytic inhibitors. Niewiarowski et al (1964) thought that the decreased fibrinolytic activity in renal disease might be explained by decreased production of urokinase or other fibrinolytic activators in the damaged kidney. Isacson & Nilsson (1969) showed, however, that even more than one year after operation bilaterally nephrectomised patients could develop fibrinolytic activity during venous occlusion and had normal amounts of plasminogen activator in the vessel walls, as measured histochemically with Pandolfi's (1967) modification of the method of Todd (1959). These findings argue against any essential role of the kidneys in the production of plasminogen activators in the body and against the decrease of fibrinolytic activity in uraemia being due to a decreased activator content of the vessel walls. In 1965 McNicol et al proposed that the levels of the fibrinolytic activity in these patients was due to an increase in fibrinolytic inhibitors. The different inhibitors have since been much investigated. Wardle et al (1970) found high levels of α_2 -macroglobulin in 18 out of 34 patients with glomerulonephritis. Larsson et al (1971a,b) determined the different inhibitors in chronic and acute renal failure. In chronic uraemia the α_2 -macroglobulin was within normal limits in chronic uraemia. The activities of inhibitors of plasminogen activation (urokinase inhibitors), measured according to Paraskevas et al (1962), were increased in 39 out of

the 50 patients examined (Larsson et al 1971a). In 18 patients with acute renal failure the activities of these inhibitors were elevated in all but one, while the α_2 -macroglobulin was normal or subnormal (Larsson et al 1971b). It is therefore now generally accepted that the low fibrinolytic activity of the blood in uraemia is due not to impaired synthesis of activators of fibrinolysis, but to an increased concentration of, above all, inhibitors of the plasminogen activation.

Fibrin degradation products (FDP) in renal diseases.

Most investigators of FDP in renal diseases have used the tanned red cell haemagglutination inhibition immunoassay of Merskey, which detects FDP down to 0.5 μ g/ml (TRCHII) (Wardle & Taylor 1968, Rayner et al 1969, Naish et al 1970, Preston et al 1971, Haanen et al 1971b, Chirawong et al 1971, Vermynen et al 1971, 1972, McNicol et al 1971, Clarkson et al 1971, Ekert et al 1972a). Other methods include a semiquantitative precipitation tube assay (Stiehm & Trygstad 1969, Stiehm et al 1971), the staphylococcal clumping test (Hawiger et al 1970), which does not reveal D- or E-products (Marder 1971, Donati et al 1971) and various types of direct and indirect latex agglutination tests. The latex agglutination tests demonstrate D-product but not E-product (Melliger 1970, Allington 1971, Svanberg et al 1973, Semeraro et al 1973). But Semeraro et al (1973) found that a direct agglutination test using latex particles coated with D- and E-antibodies was more sensitive for detecting D- and E-products than an indirect test using particles coated with fibrinogen. Furthermore, their direct latex agglutination test was more sensitive for detecting D- and E-products than for detecting clottable, high molecular weight products. The quantitative and specific immunochemical method of Niléhn (1967a, 1971) has been used by Larsson et al (1971c) and Hedner & Nilsson (1971).

FDP in urine have been determined with the same methods in both unconcentrated urine (Larsson et al 1971c, Clarkson et al 1971, Preston et al 1971, Ekert et al 1972a) and in 24-hour samples concentrated 20-1000 times by ultrafiltration, lyophilisation or dialysis against polyethylene glycol (Braun & Merrill 1968, Stiehm et al 1971, Clarkson et al 1971, Cash & Clarkson 1971, Vermynen et al 1971, Ekert et al 1972a). The urinary FDP have been typed by different methods including qualitative methods, such as immunoelectro-

phoresis and immunodiffusion (Braun & Merrill 1968, Antoine et al 1969, Zühlke & Hermann 1970, Haanen et al 1971b); and by comparisons of the TRCHII and the staphylococcal clumping test which determines only HMWDP (Vermylen et al 1972). More specific and sensitive test for typing FDP are the immunochemical methods used by Rayner et al (1969). The different kinds of FDP in serum and urine have also been separated by chromatography on DEAE-cellulose (Niléhn 1967, Nussen-zweig 1961a) and by gel filtration on Sephadex G-200 (Niléhn 1967, Marder & Sherry 1969a, Clarkson et al 1971).

FDP in uraemia. FDP occur in serum most often in acute renal failure (Wardle & Taylor 1968, Stiehm & Trygstad 1969, Larsson et al 1971b, Hedner & Nilsson 1971, McNicol 1971, Ekert et al 1972, Briggs et al 1972). Thus, FDP were found in the serum from 14 out of 18 patients with acute renal failure. Unconcentrated urine samples from 14 of these patients were examined for FDP which were found in 8. The FDP concentration was usually higher in urine than in serum (Larsson et al 1971b). The FDP concentration in both varied with the activity of the disease (Braun & Merrill 1968, Stiehm & Trygstad 1969, Hedner & Nilsson 1971, Larsson et al 1971c, Clarkson et al 1971, Preston et al 1971) and is, therefore, a valuable indication of the course of the disease and of the effect of therapy (Stiehm & Trygstad 1969, Larsson et al 1971d).

In chronic uraemia FDP have been demonstrated more often in urine than in serum (Rayner et al 1969, Preston et al 1971, Briggs et al 1972). Of 76 patients with chronic uraemia, FDP were found in the serum of 28 (37 %) and in the unconcentrated urine of 12 (39 %) out of 31 patients (Hedner & Nilsson 1971). Again the FDP concentration varied with the degree of activity of the disease (Hedner & Nilsson 1971, Larsson et al 1971b, Clarkson et al 1971, Preston et al 1971). In recent years interest has centered on FDP in the urine, which have been correlated with the histological abnormalities (Clarkson et al 1971, Chirawong et al 1971).

FDP in glomerulonephritis. FDP in the serum as well as in the urine have been found predominantly in the proliferative types of glomerulonephritis and most often in the acute forms of this type of nephritis, and less

often in membranous glomerulonephritis or in minimal renal lesions (Naish et al 1970, Cash & Clarkson 1971, Clarkson et al 1971, Chirawong et al 1971, Stiehm et al 1971, Preston et al 1971, Shah et al 1972, Ekert et al 1972a). Clarkson et al (1971) found FDP in the urine from 79 out of 108 patients with proliferative glomerulonephritis and from 10 out of 12 with membranoproliferative glomerulonephritis. The concentrations were higher in the proliferative cases. All the patients with glomerulonephritis of Ekert et al (1972a) had FDP in urine but only some of them had FDP also in serum. The frequency of FDP was correlated with the extent of fibrin deposits in the kidney (Stiehm & Trygstad 1969, Chirawong et al 1971, Clarkson et al 1971).

Urinary FDP in glomerulonephritis have been typed by Vermynlen et al (1972) and Clarkson et al (1971). Both groups found predominantly low molecular weight fragments. Clarkson et al (1971) distinguished between proliferative forms of glomerulonephritis, in which they found low molecular fragments in the urine, and membranous forms including those with minimal changes in the kidneys, in which urine contained high molecular weight fragments in low concentrations.

FDP after renal transplantation. FDP after renal transplantation have been found in the serum during the first postoperative weeks and during rejection episodes (Antoine et al 1969, Carlsson et al 1970, Haanen et al 1971, Bennett et al 1972). Urinary FDP have always been found during the first 2 weeks after renal transplantation and ascribed to ischaemic damage to the kidney (Braun & Merrill 1968, Antoine et al 1969, Clarkson et al 1970, Carlsson et al 1970, Cash & Clarkson 1971, Grob et al 1971). The appearance and later increase of FDP are an early sign of rejection (Braun & Merrill 1968, Antoine et al 1969, Zühlke & Hermann 1970, Carlsson et al 1970, Clarkson et al 1970, Grob et al 1971, Haanen et al 1971b, Shah et al 1972, Naish et al 1973).

Typing of urinary FDP after renal transplantation has given variable results, some having found low molecular weight fragments (Braun & Merrill 1968, Zühlke & Hermann 1970, Haanen et al 1971b), and others both low and/or high molecular weight fragments (Antoine et al 1969, Grob et al 1971).

FDP in patients with disseminated intravascular coagulation. In experimental animals and in human patients

disseminated intravascular coagulation without renal impairment causes the appearance of D- and E-products in urine. When the kidneys are involved, high molecular weight degradation products (HMWDP) are found (Herschlein & Steichele 1968, Rayner et al 1969, Preston et al 1971). In one of Herschlein & Steichele's (1968) patients with disseminated intravascular coagulation caused by placental abruptio placentae, FDP were demonstrable in the urine after they had disappeared from the serum.

In summary, therefore, repeated determination of urinary FDP has proved more informative than determination of serum FDP in renal diseases and in the diagnosis of threatening rejection of renal transplants. The amount of urinary FDP varies with the amount of fibrin deposited in the kidney. Urinary FDP have also been found in urine from patients with glomerulonephritis, most often of the proliferative type. Patients with disseminated intravascular coagulation have FDP in the serum but not in the urine unless they have co-existing renal lesions.

The purpose of the present investigation was to study the origin and significance of FDP in renal diseases. This has been done by typing of the urinary FDP in patients with early glomerulonephritis, uraemia, after renal transplantation and during thrombolytic therapy (I, II, III). The concentrations of FDP in the urine have been compared with those of other proteins, viz albumin, lysozyme, α_2 - and β_2 -microglobulin, which depend on glomerular (albumin) and tubular function (α_2 - and β_2 -microglobulin) respectively (II).

I. Typing of fibrinogen degradation products in urine in various clinical disorders

The material consisted of urine specimens from 34 patients (12 with chronic uraemia, 3 with acute uraemia, 11 examined after kidney transplantation, 6 during thrombolytic therapy, 2 with haematuria) and sera from 17 patients with various conditions.

An immunochemical method based on Laurell's (1966) rocket method was used for typing FDP in urine and serum. The serum was obtained from blood collected with EACA. EACA was unnecessary when urine was collected from patients with uraemia, such patients having no urokinase activity in the urine (Vreeken et al 1966, Carlsson et al 1970). Urine (dil 1/1) or serum (dil 1/1) was applied to separate agarose gel plates containing antisera to human fibrinogen, D-product and E-product respectively. On high voltage electrophoresis the antigen is forced into the gel. Precipitation peaks form the heights of which are proportional to the amount of antigen in the sample. Fibrinogen is used as a standard for all the plates. A mixture of different types of FDP forms two or three peaks because of differences in molecular weight, charge, and antigenic determinants. By demonstrating single or double peaks against the different antisera it is possible to decide the type or types of FDP and their concentration in relation to fibrinogen. Addition of a substance identical with that present in the urine results in an increase in the height of the peak. If the substance added is not identical, the result is a double peak.

FDP were determined also after addition of thrombin (20 N.I.H. units/ml) or protamine sulphate (1 % solution 0.2 ml/ml) to the urine samples or after heating (60°C; 30 min) and the results were compared with those obtained without such treatment.

Chronic uraemia (Table I). The patients had FDP in the urine in concentrations ranging from 10 to 120 $\mu\text{g/ml}$. In all 12 patients they consisted mainly of high molecular weight degradation products (HMWDP). One patient also had E-product in low concentration.

Acute uraemia (Table I). All the FDP in the urine were

HMWDP in concentrations of 30 to 120 $\mu\text{g/ml}$.

Renal transplantation (Table I). The concentrations of FDP in the urine varied from 5 to 1000 $\mu\text{g/ml}$. All the patients had HMWDP and one also had D-products, though in low concentration.

Thrombolytic therapy (Table I). 20 out of 28 patients examined during thrombolytic therapy had FDP in the urine (10-200 $\mu\text{g/ml}$). In 6 of the patients the urinary FDP were typed and found to be D- and E-products. Serum creatinine and creatinine clearance were normal in all the patients.

Haematuria. In 2 haemophilic patients the urine contained fibrinogen in low concentration (10 and 20 $\mu\text{g/ml}$). One of the patients also had E-product.

FDP in the serum were typed in 17 patients with various diseases. The FDP concentration in the serum varied between 40 and 380 $\mu\text{g/ml}$. All but 4 had HMWDP, and 9 out of these 13 also had D- and E-products. The other 4 patients had only D- and E-products.

Table I

Types of FDP in urine from different categories of patients. HMWDP = high molecular weight degradation products. FDP = fibrin/fibrinogen degradation products

Diagnoses (no. of patients)	Type of FDP in the urine (Bouma et al 1971)		Range of FDP in the urine - $\mu\text{g/ml}$ (Nilēhn 1967)
Chronic uraemia (12)	HMWDP (11)		10-120
	- " - +E (1)		
Acute uraemia (3)	HMWDP (3)		30-120
After renal trans- plantation (11)	HMWDP (10)		5-1000
	- " - +D (1)		
During thromboly- tic treatment (6)	D + E (5)		10-200
	D (1)		

II. Urinary fibrinogen / fibrin degradation products (FDP) in renal diseases and during thrombolytic therapy

24-hour urine volumes from 10 apparently healthy persons, 10 patients with chronic uraemia, 4 patients examined at various intervals after renal transplantation, and 12 patients receiving thrombolytic therapy were collected and concentrated until the protein concentration was about 7 g/100 ml (Biuret method). The urinary FDP were measured and typed with the method of Bouma et al (1971; I). Gel filtration on Sephadex G-200 or ion-exchange chromatography was performed on some of the samples. FDP in the fractions were determined in the way described in I. Plasminogen, lysozyme, albumin, α_2 - and β_2 -microglobulin were determined in the urine, as well as serum creatinine and creatinine clearance.

Normal urine. The concentrated normal urine contained no FDP, plasminogen or lysozyme, and less than 50 mg albumin per 24 h. The urokinase activity was adsorbed on bentonite. Different types of FDP were then added, each to a final concentration of 0.6 g/l. After gel filtration (Fig. 2) the HMWDP were found in one peak around fraction No. 15 (45 ml effluent volume; K_d 0.01-0.03). When only D- and E-products were added there were two peaks, not entirely separate (K_d values 0.18-0.22 and 0.28-0.32, respectively).

Uraemia. All the patients had HMWDP in the urine, 2 of them also had D-products and 2 others, also E-products. After gel filtration on Sephadex G-200 before and after treatment of the urine with thrombin the FDP were present in the high molecular weight peak (K_d 0.01). In 3 of the urines this peak decreased markedly after treatment with thrombin, indicating the presence of coagulable X-products together with Y-products (Fig. 2). Gel filtration also showed low molecular weight degradation products, in all urines although in extremely low concentrations (K_d 0.13-0.22). Neither lysozyme nor albumin or plasminogen varied with the FDP concentration in the urine.

Renal transplantation. 3 out of 4 patients had HMWDP in the urine. After gel filtration, FDP were present

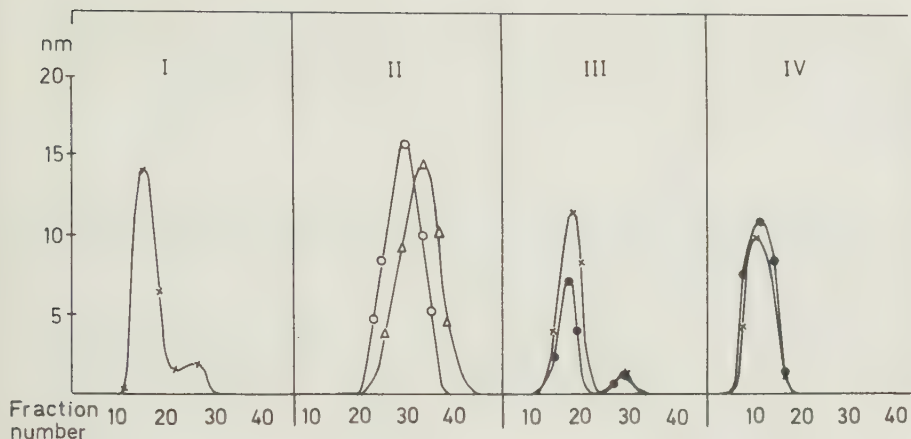


Fig. 2 Gel filtration on Sephadex G-200 of normal urine to which had been added HMWDP (I), D- and E-products (II), urine from a uraemic patient (III) and urine from a haemophilic patient with a sclerosing glomerulonephritis (IV). The peak heights on agarose gel plates in mm obtained when testing the fraction against anti-fibrinogen (x—x), anti-D (o—o) and anti-E (Δ—Δ) are given. The pattern obtained after thrombin treatment (●—●) of the urine are given in III and IV

in the high molecular weight zone (Kd 0.02-0.03). One of the patients had X-products together with Y-products, and in 2 out of the 3 cases also low molecular weight products (Kd 0.20-0.23) but again in very low concentrations.

The fourth patient in whom renal function appeared normal (i.e. normal serum creatinine and creatinine clearance) had only E-product in the urine and no plasminogen, lysozyme or albumin.

Thrombolytic therapy. In the 12 patients on thrombolytic therapy, the urine contained only low molecular weight FDP. Gel filtration on Sephadex G-200 gave Kd values of 0.13-0.21. Chromatography on DEAE-cellulose gave two separate peaks one reacting with anti-D-serum and the other with anti-E-serum. In 4 of the patients urinary lysozyme and α_2 - and β_2 -microglobulin were increased, but albumin and serum creatinine were normal.

III. Urinary fibrin/fibrinogen degradation products (FDP) and glomerulonephritis

The clinical material consisted of 24 patients with various forms of glomerulonephritis. The disease was classified according to Bruhn (personal communication) on the basis of the light microscopic appearance of fine needle aspiration biopsy specimens of the kidney. In all the patients serum creatinine levels and endogenous 24-hour creatinine clearance were normal. 24-hour urine volumes were collected without addition of a fibrinolytic inhibitor and the urine was concentrated 20-1000 times against a membrane that retained proteins with a molecular weight of more than 12,000. FDP were determined in serum and urine by the method of Niléhn (1967a). Urinary FDP were determined and typed before and after concentration of the urine. No FDP could be demonstrated in 24-hour urine volumes of 19 control subjects.

Proliferative glomerulonephritis (Table II). None of the 19 patients with proliferative glomerulonephritis had any FDP in serum or unconcentrated urine. Concentrated 24-hour urine samples from 18 of the 19 patients did contain FDP (up to 1,650 $\mu\text{g}/24$ hrs). The urinary FDP were typed in 12 of the samples and were found to be HMWDP in all of them. The 12th contained only D- and E-products. This patient had the highest urokinase activity in the urine (11 Ploug units/ml); in the other patients it was ≤ 6 Ploug units/ml (normal > 8 Ploug units/ml).

None of the 19 patients had plasminogen or α_2 -macroglobulin in the urine, while the albumin concentrations varied between < 50 and $3,578$ mg/24 hrs.

The patients with proliferative glomerulonephritis had proteinuria of selective glomerular type with excretion of predominantly albumin and transferrin, but no immunoglobulins.

Membranous glomerulonephritis (Table II). None of the 5 patients with this type of glomerulonephritis had FDP in the serum. One had FDP in unconcentrated urine (35 $\mu\text{g}/\text{ml}$) and another 2 in concentrated 24-hour volume (30 and 68 $\mu\text{g}/24$ hrs). All these were HMWDP.

The remaining 2 patients had no FDP even in the concentrated urine.

None of the patients had plasminogen or α_2 -macroglobulin in the urine but albumin in concentrations ranging from <50 to 3,843 mg/24 hrs. One of the patients without urinary FDP was the only one with a normal albumin concentration (<50 mg/24 hrs) whereas the other without urinary FDP had the highest urinary albumin concentration of all (3,384 mg/24 hrs). In all 5 patients, urinary urokinase activity was decreased (1-3 Ploug units/ml).

The patients with the membranous type of glomerulonephritis had unselective proteinuria with excretion also of high molecular weight proteins.

It can be seen, therefore, that patients suffering from severe renal diseases with uraemia and patients with incipient proliferative glomerulonephritis had predominantly high molecular weight FDP in their urines (I, II, III). Smaller amounts of FDP were present in the urines of only some of the patients with incipient membranous glomerulonephritis although the renal damage was more severe than in proliferative glomerulonephritis, as shown by the unselective proteinuria (III). In patients with pyelonephritis concentrated 24-hour urine volumes contained only low concentrations of FDP (Table II). Neither in patients with uraemia nor in those with incipient glomerulonephritis was there any correlation between urinary concentrations of albumin, plasminogen or lysozyme and that of FDP (Table III) (II, III).

The urokinase activity was decreased in the urine also of the patients with incipient glomerulonephritis (III). Although plasminogen was present in the urine of uraemic patients there was no degradation of the HMWDP (II).

In patients after renal transplantation the urine contained predominantly HMWDP, at least during rejection episodes (I, II). The urine from one patient without any symptoms or signs of renal impairment (II) contained E-product. Low molecular weight degradation products were present in the urine samples from about 70 % of patients receiving thrombolytic therapy (I, II). In these renal function tests were normal but urinary

levels of lysozyme, α_2 - and β_2 -microglobulin were raised.

Table II

FDP in serum and urine, the type of urinary FDP and the urokinase concentration in urine from patients of different categories

Diagnosis (no. of patients)	FDP		FDP type	Urokinase activity in urine Ploug U/ml
	in serum $\mu\text{g/ml}$	in urine $\mu\text{g/24 h}$		
Incipient glomerulo- nephritis				
proliferative glomerulo- nephritis (19)	0	90-1,650	X+Y (17) D+E (1)	0.9-6
membranous glomerulo- nephritis (5)	0	30, 68	X+Y	1-3
Pyelonephri- tis (8)	0	28-300	HMDP	1-5
Controls (19)	0		-	>6

Table III

Urinary excretion (24 h) of different proteins in
patients with uraemia of various aetiologies

Case	FDP mg/24 h	Albumin mg/24 h	Lysozyme mg/24 h	Plasminogen %
1	2.8	638	1.4	86
2	3.2	350	-	-
3	1.0	-	2.7	31
4	0.2	240	1.3	110
5	0.9	480	2.4	-
6	1.4	1,200	5.0	136
7	5.2	48	5.4	1
8	1.1	200	2.7	128
9	0.1	4	<3	0
10	5.0	93	0.4	100

DISCUSSION

In renal diseases, FDP may appear in the urine by 1) filtration of FDP from the blood, because of (a) increased concentration of FDP in the serum (b) increased glomerular permeability, (c) an insufficient tubular protein reabsorption, depending on impaired function of the tubular cells or overloading of the normal tubular reabsorption mechanism; 2) further degradation in the lower urinary tract of fibrinogen filtered through glomeruli; or 3) local lysis of fibrin/fibrinogen mediated by the fibrinolytic activators present in the kidneys (Yatzidis 1964, Naish et al 1970, Clarkson et al 1971, Preston et al 1971, Hedner & Nilsson 1973).

In considering the origin of urinary FDP the molecular weight of the fragments has to be taken into account. As pointed out above, no unanimity has been achieved on this point. Some have demonstrated two low molecular weight fragments (Braun & Merrill 1968, Zühlke & Hermann 1970, Clarkson et al 1971, Haanen et al 1971b, Vermynen et al 1972); others have found only high molecular weight fragments (Antoine et al 1969) and yet others have found both (Grob et al 1971). Using a specific immunochemical method for typing FDP Rayner et al (1969) found HMWDP in the urine only in patients with uraemia and after renal transplantation. A quantitative immunochemical method for typing FDP has been described in I. Using this method as well as gel filtration on Sephadex G-200 HMWDP were shown to occur in the urine from patients with both chronic and acute uraemia and after renal transplantation (I, II), while only D- and E-products were found during thrombolytic therapy (I, II). This is in accord with experimental results (Rayner et al 1969) showing that intravenously infused D- and E-fragments are cleared from the blood while HMWDP are not. Using gel filtration on Sephadex G-200 Clarkson et al (1971) claimed that the urine from patients with proliferative glomerulonephritis contains D- and E-fragments, whereas patients with minimal lesions show traces of HMWDP. This could not be confirmed by the results presented in II and III. The urine of uraemic patients contains little or no urokinase (Smyrniotis et al 1959, Edward et al 1964, Vreeken et al 1966, Carlsson et al 1970). The observations made support the conclusion that a decrease in the urokinase activity in the urine is an early sign

1973a, Mills 1972, Furlan & Beck 1972, Marder et al 1972, Gaffney & Brasher 1973). The heterogeneity of the D-fragment has been thoroughly studied by several authors (Gaffney 1972b,c, Gaffney & Brasher 1973, Pizzo et al 1973b). The D-fragment derived from cross-linked fibrin, called D-dimer, was found to migrate like IgG and fragment Y in a SDS-acrylamide system (Gaffney 1972b,c, Gaffney & Brasher 1973). Gaffney (1972c) also found that the D-fragment obtained on plasmin digestion of purified fibrinogen contained two types (mol.wts 82,000 and 73,000) differing in the length of the γ -chain. The D-fragment obtained from plasma contained mainly the type with a molecular weight of 82,000 together with some fragments with slightly higher molecular weight. The heterogeneity of fragment D has been further studied by Furlan & Beck (1973) and Mosesson et al (1973). Mosesson et al (1973) suggested that one type of D-product originates from each fibrinogen molecule, which might explain the variety of D-products in a plasmin digest of fibrinogen. Furlan & Beck (1973) have isolated a fragment with a molecular weight of 46,000 and with partial immunological identity with respect to fragment D, which they called fragment d. Fragment d was found to appear early during plasmin digestion and not to form complexes with fragment E. Neither it was a dimer of fragment D. Catanzaro et al (1971) found 8 charge isomers of fragment D on acrylamide gel electrophoresis. Using I125 fragment D, they found two separate rates of plasma clearance viz 95.3 ± 4.1 of the fragment D was cleared with a $T/2$ of 2.2 ± 0.4 hrs and 4.7 ± 4.7 with a $T/2$ of 33.2 ± 4.7 hrs. The E-fragment derived from fibrinogen is identical with that derived from fibrin (Taylor et al 1972). The D- and E-fragments are linked together (Budzynski et al 1967, Edgington & Plow 1972). The relative D- and E-content of such a bi-molecular complex has been discussed. It has been assumed that only one molecule of fragment E can be derived from one molecule of fibrinogen (Marder et al 1969a, Wallén 1971). Edgington & Plow (1972) have, however, recently contended that the two fragments combine in a 1:1 molar ratio and that one D:E is derived from each half of the fibrinogen molecule. They also produced evidence for a neoantigen of fibrinogen situated in the D-fragment exposed by the cleavage process. Neoantigens specific for fibrinogen D-fragment or fibrin D-fragment were found. These neoantigens make it possible to distinguish between the fragment D derived from fibrinogen and that derived from fibrin (Plow & Edgington 1972, 1973). Available methods for determining FDP determine all fragments containing antigenic deter-

of renal disease (III), despite the fact that one of the 24 patients with incipient glomerulonephritis had normal urokinase activity and low molecular weight degradation products in the urine. Some patients in the material of Clarkson et al (1971) may also have had a normal urokinase activity in the urine and further degradation of the products in the lower urinary tract may therefore, have occurred.

Several workers have pointed out that in renal diseases there is no correlation between the presence or concentration of FDP in serum and in urine. The concentration of FDP in urine is usually higher than in serum (Hedner & Nilsson 1971, Larsson et al 1971c, Preston et al 1971, Stiehm et al 1971, Vermynen et al 1971, McNicol et al 1971, Ekert et al 1972a). None of the patients with incipient glomerulonephritis described in III had FDP in serum, although almost all of them had FDP in the 24-hour urine volume, while 24-hour urine specimens from normal individuals did not contain FDP (II, III). Furthermore, the concentration of the urinary FDP is independent of the severity of proteinuria and its selectivity (Clarkson et al 1971, Ekert et al 1972a, Vermynen et al 1972). It was also shown in II and III that neither the lysozyme nor the albumin or plasminogen concentration varied with the FDP concentration in the urine (Table III). FDP in urine occurred more common and in higher concentrations in patients with proliferative glomerulonephritis, although these patients had a selective type of proteinuria, excreting mainly albumin. In contrast, in patients with membranous glomerulonephritis urinary FDP were less common and occurred in lower concentrations although all the patients had unselective proteinuria with excretion of high molecular weight proteins (III). These findings argue against the possibility that FDP are in the urine simply by being filtered from the serum through the damaged glomeruli.

The high molecular weight of urinary FDP suggests that tubular reabsorption plays no important role in the regulation of their excretion in renal diseases. Ekert et al (1972a) have pointed out that in patients with the Fanconi syndrome, tubular proteinuria is not necessarily associated with large amounts of urinary FDP. Reabsorption by the tubular cells of the low molecular weight degradation (D- and E-) products, may, however, be significant. The finding (II) of α_2 - and β_2 -microglobulin and increased amounts of lysozyme in the urine from patients with normal renal function receiving

thrombolytic therapy in association with high urinary concentrations of low molecular weight degradation products, suggest overloading of tubular reabsorption capacity. Low molecular weight degradation products may therefore be present in the urine without any sign of renal disease. This conclusion was confirmed by the finding of E-products in a patient with no signs of renal impairment when examined 5 months after renal transplantation (II).

Further degradation of fibrinogen filtered through damaged glomeruli requires the presence of a fibrinolytic activator in the urine. Normal urine contains high concentration of urokinase (Vreeken et al 1966, Boomgaard et al 1969) which is probably synthesised in the kidney (Åstedt & Pandolfi 1972). In renal diseases the urokinase activity decreases or disappears (Vreeken et al 1966, Carlsson et al 1970) already in an early phase (III). However, in patients with incipient glomerulonephritis urokinase activity is sometimes normal (III). Low molecular weight fragments in the urine from such patients may result from further degradation of HMWDP or fibrinogen in the lower urinary tract.

Urinary FDP were found to be more common and to occur in higher concentrations in patients with proliferative glomerulonephritis (III), in which fibrin deposits are more extensive (Clarkson et al 1971, Chirawong et al 1971). Fewer patients with membranous glomerulonephritis had FDP in the urine and then in low concentrations (III). These findings suggest that in patients with renal diseases urinary HMWDP are to a great extent caused by local lysis of intrarenal fibrin, mediated by plasminogen activators in vascular endothelium and glomeruli in the diseased kidneys and released from the endothelial cells into the bloodstream. However, an additional effect of small amounts of urokinase in the glomeruli cannot be excluded. The urine from two patients with severe haemophilia A and renal disease contained HMWDP (Fig. 2). Both patients were nephrotic and had sclerosing glomerulonephritis. It seems less probable that much fibrin will be deposited in the kidneys of such patients who have severe defects in the coagulation mechanism. The products are in these patients thus most probably derived from partial degradation of circulating fibrinogen in the renal vessels and only to a lesser extent fibrin deposits in the kidney. The presence of clottable X-products in the urine of some patients with renal diseases and in some of those examined after renal transplantation (II)

favours the view that such a mechanism contributes to the urinary HMWDP also in the absence of coagulation defects. This hypothesis is supported by the very low concentrations of FDP in 24-hour urine concentrates of patients with membranous type of glomerulonephritis (III) or pyelonephritis (Table II) (Shah et al 1972).

Considerations on treatment of renal diseases with FDP.

In 1964 Vasalli & McCluskey showed in animal experiments that the deposition of fibrin following experimental renal damage could be prevented by pretreatment of the animals with warfarin. Similar effects, especially in acute glomerulonephritis and haemolytic-uraemic syndrome, have been reported for heparin (Künzler & Aalam 1964, Brian et al 1967, Kincaid-Smith et al 1968, Kincaid-Smith 1969, Stiehm & Trygstad 1969, Gilchrist & Lieberman 1969, Gilchrist et al 1969, Uttley 1970, Herdman et al 1970, Luke et al 1970, Larsson et al 1971d, Wardle & Uldall 1972, Fuller et al 1972, Arieff & Pinggera 1972, Cade et al 1973). Complicating haemorrhages have, however, occurred (Kincaid-Smith et al 1968, Katz et al 1969) although the course of rapidly progressive glomerulonephritis appeared to be arrested (Arieff & Pinggera 1972). Wardle & Uldall (1972) reported that heparin improved a few patients during rejection episodes of renal grafts but not patients with proliferative glomerulonephritis; in this disease it was suggested that heparin should be used to supplement other drug such as cytostatics and steroids.

Dipyridamol, an inhibitor of platelet aggregation, has been used successfully by Kincaid-Smith et al (1968, 1970) together with heparin and phenindion in acute renal failure, malignant hypertension and glomerulonephritis. Indomethacin, another inhibitor of platelet aggregation, has also been used by itself in the treatment of proliferative glomerulonephritis with good effects (Vermylen et al 1970, 1972). A combination of streptokinase and heparin or aspirin (Monnens & Schretlen 1968, Sutor et al 1972) has been used in haemolytic-uraemic syndrome. Thus agents which counteract either platelet aggregation or coagulation appear to be of value in the treatment of various renal diseases. Determination of urinary FDP may help to establish in which cases these agents are indicated, and to follow the course of treatment.

It is concluded from the present investigation that HMWDP occurring in the urine in renal diseases may be derived from partial degradation of fibrin deposited in the kidney and/or circulating fibrinogen. The degradation is probably mediated by released plasminogen activators and by small amounts of urokinase in the glomeruli. The HMWDP are excreted into the urine through damaged glomerular basement membrane. Further degradation in the lower urinary tract may occur in patients with normal urokinase concentration, resulting in the formation of D- and E-fragments.

Determination of FDP in unconcentrated urine and in 24-hour urine concentrates is of diagnostic value also in the early stages of renal diseases; for following their course and for assessing therapy.

B. Activator inhibitor in human serum

Inhibitors of fibrinolysis fall into two groups, one acting like antiplasmin and one inhibiting plasminogen activation. Different antiplasmins have been identified, namely 1) α_2 -macroglobulin (mol.wt 800,000) (Shulman 1952, Brown et al 1954, Norman & Hill 1958, Schultze et al 1963, Mehl et al 1964, Weiss 1965, Jacobsson 1965, Steinbuch et al 1965, 1972, Ganrot & Scherstén 1967, Ganrot 1967b); 2) α_1 -antiplasmin (mol.wt 47-60,000) (Shulman 1952, Jacobsson 1955, Norman 1958, Moll et al 1958, Bundy & Mehl 1959, Schultze et al 1962, Rimon et al 1966, Heimbürger 1967) and 3) inter- α -inhibitor (Steinbuch & Loeb 1961, Heide et al 1965, Heimbürger & Haupt 1965, Steinbuch et al 1966, Schwick et al 1966) (mol.wt 180,000), which is probably the same as the protein π described by Steinbuch & Loeb (1961) and Steinbuch et al (1966).

The existence of an inhibitor of plasminogen activation was suggested as early as 1937 by Schmitz and subsequently by Hultin & Lundblad (1949) and Lewis & Ferguson (1951). Müllertz (1954a) discovered that bovine globulin inhibited the conversion of bovine plasminogen to plasmin by an activator prepared by the action of streptokinase on human globulin. In 1960 Flute demonstrated an inhibitor in human serum which was active against streptokinase activation of plasminogen but not against plasmin itself. Nilsson et al (1961b) and later Brakman et al (1966) reported an increased inhibition of plasminogen activation in patients with severe thrombotic disease.

But no unanimity has been achieved regarding such inhibitor activity in serum (Paraskevas et al 1962, Jacobsen 1964, Dudok de Wit 1964, Bennett 1967, 1970, den Ottolander et al 1969a). This is due partly to methodological difficulties. An activator inhibitor in normal serum has repeatedly been demonstrated by means of clot lysis methods (Müllertz 1954a, Nilsson et al 1961b, Hawkey & Stafford 1964, Bennett 1967, 1970, den Ottolander et al 1969a,b). Using a fibrin plate method Brakman et al (1966) found an inhibitor against tissue activator in some patients with thrombotic diseases. With I^{131} -labelled clots Dudok de Wit (1964) found no inhibition of urokinase activation of plasminogen in

plasma. Jacobsen (1964) likewise failed to demonstrate any such inhibition in a caseinolytic system, whereas McNicol et al (1963) reported activator inhibition in a similar system. Activator inhibitor in normal serum and its pattern after gel filtration has also been described by Aoki & v.Kaulla (1971b). Although Paraskevas et al (1962) and Bennett (1967) pointed out that the clot lysis systems used by them measured predominantly the antiactivator level in serum, it is likely that none of these methods completely exclude inhibitory effects of antiplasmin substances. Lauritsen (1968) has elaborated a caseinolytic system in which an antiplasmin effect is claimed to be excluded. Serum samples are added to a system comprising porcine plasminogen, which is activated by urokinase. After a certain activation time EACA is added and the amount of plasmin formed is measured by caseinolysis. The antiplasmin effect is determined separately by adding the serum samples after the activation of the plasminogen has been stopped by EACA. The difference between the two assays is a measure of the activator inhibitor present in the serum. With this method (Lauritsen 1968) urokinase inhibitor was found in 38 normal persons. He pointed out that the activator inhibitor will be slightly underestimated by this caseinolytic method because of the plasminogen in the serum. However, the amount of plasminogen is small compared with the amount of plasminogen added. High plasminogen levels in serum can mask the activity of the activator inhibitor (Paraskevas et al 1962).

Thus, there is good evidence for the existence of an inhibitor of the plasminogen activation in human serum but little is known about its chemical nature and properties.

Properties of the activator inhibitor. The activator inhibitor of human serum is an α_2 -globulin (Nilsson et al 1961b). It is destroyed by heating at 56°C for 30 minutes. The activity decreases markedly also after storage at room temperature or at +4°C for more than 24 hours. Acidification to pH 2.0 destroys the activity (Nilsson et al 1961b, Paraskevas et al 1962, McNicol et al 1963, Bennett 1967). The inhibitor is not dialysable and not adsorbed on BaSO₄ (Nilsson et al 1961b). Alimentary lipaemia has no demonstrable effect on the activity of urokinase inhibitors (Paraskevas et al 1962, Gormsen 1962, Bennett 1967). No sex

difference was found by Paraskevas (1962) or Gormsen (1962), whereas Mann (1967) found higher concentrations in women than in men. Lauritsen (1968), and Blix (1964) found as much urokinase inhibitor in serum as in plasma whereas Bennett (1970) reported shorter lysis times for plasma containing clots than for serum containing clots. The activator inhibitor level varies independently of those of α_2 -macroglobulin or α_1 -antiplasmin (Paraskevas et al 1962, Bennett 1967). The inhibitor described by Aoki & v.Kaulla (1971b) inhibited plasminogen activator obtained from the walls of human blood vessels both immediately and progressively. The molecular weight was about 80,000 and the inhibitor was immunochemically distinct from α_2 -macroglobulin.

Activator inhibitor in various diseases and during pregnancy. In patients with severe thrombotic diseases the level of activator inhibitors is raised and this has been put forward as an aetiological factor in such diseases (Nilsson et al 1961b, Brakman et al 1966). But Hedner & Nilsson (1971; IV) found the activity of the activator inhibitors to be increased in only 19 % of patients with recurrent deep venous thrombosis. This suggest that increased activity or activator inhibitor could only rarely be involved as a cause of thrombotic disease.

Increased levels of activator inhibitor in systems containing urokinase or streptokinase have been found in several other clinical conditions including malignant diseases (Gormsen 1962), myocardial infarction (Bennett et al 1967, Thorsen 1973), and surgical injury (Olow 1964, Bennett 1967). In atherosclerosis the results are conflicting: Gormsen (1962) found activator inhibitor within normal limits whereas Cucuianu et al (1972) reported it increased. Increases in the urokinase inhibitors have also been found during the last trimester of pregnancy (Brakman & Astrup 1963, Kawano et al 1968, Lauritsen 1969b, Thorsen 1973), but not by others (Correll & Sjoerdsma 1962, Bonnar et al 1969, Nilsson & Kullander 1967).

The activity of the activator inhibitors has been reported as low (Gormsen 1962, Cucuianu et al 1972) or unchanged (den Ottolander et al 1969b) in patients with liver cirrhosis.

Activator inhibitor activity in different organs. Urokinase activation of plasminogen is inhibited by extract of placenta (Kawano et al 1968, Uszynski et al 1969). Recently Åstedt et al (1972) demonstrated that urokinase activity is inhibited by human placenta explants cultured in medium containing urokinase. These explants also inhibited the fibrinolytic activity of explants of kidney and aorta. The inhibition occurred in the medium. These experiments indicate that the activator inhibitor of human placenta inhibit urokinase as well as fibrinolytic activators released from vessel and kidney explants.

The urokinase inhibitor of placenta has been purified (Kawano et al 1970, Uszynski & Abildgaard 1971); the product does not inhibit tissue activator prepared from human heart (Kawano & Uemura 1971). The placental urokinase inhibitor (Kawano et al 1970) can be separated into two fractions by gel filtration, a minor one with a molecular weight of 70,000 and a major one with a molecular weight of 43,000 (Kawano et al 1970). The inhibitor fraction was stable at +2°C for 12 months as well as stable to acid (pH 3). The inhibition produced by this material was a progressive one. In some respects the urokinase inhibitor described by Uszynski & Abildgaard (1971) was similar; it inhibited only the urokinase activation of plasminogen in a progressive manner. This was an α_1 -globulin, the major fraction of which had a molecular weight of 100,000. The discrepancy was ascribed to the inhibitor of Kawano et al (1970) having been exposed to acid which might have caused some dissociation of the inhibitor.

It has thus been shown that human serum and placenta contain activator inhibitors.

The purpose of the present investigation was to elucidate the activator inhibitor in human serum, its relationship to the antiplasmins, and its variation in different diseases, especially in uraemia, in individuals with congenital high proteolytic capacity, in morbus thromboticus and during pregnancy (IV, V, VI, VII, IX). The activator inhibitor was further studied after partial purification from normal human serum with respect to molecular weight and physical and immunochemical properties.

I V. U r o k i n a s e i n h i b i t o r s i n s e r u m i n a c l i n i c a l s e r i e s

The clinical material consisted of 1,008 patients; with the following conditions: 304 with different types of liver diseases (cirrhosis, 174; hepatitis, 22; primary cancer, 24; secondary cancer, 50; miscellaneous, 35), 109 with renal diseases (chronic uraemia, 70; acute uraemia, 14; renal and urinary tract diseases without uraemia, 10; after renal transplantation 15), 148 with deep venous thrombosis examined at least 3 months after the last thrombotic episode, 144 with leukaemia (chronic leukaemia, 72; acute, 72), 76 with other malignant diseases, 158 with myelomatosis, 40 pregnant women in the third trimester and 29 women on contraceptives (low dose gestagen).

The inhibitors of the urokinase activation of plasminogen, α_2 -macroglobulin and α_1 -antiplasmin were determined in serum.

High levels (>140 %) of the urokinase inhibitors were most common in patients with renal or malignant diseases; namely in 59 out of 70 patients with chronic uraemia; all 14 patients with acute uraemia; in all 15 patients examined after renal transplantation; and in half of the patients with malignant diseases (35 out of 76). Some of the patients with widespread metastases had extremely high values (>500 %).

Urokinase inhibitors were reduced in comparatively few patients; namely in 5 out of 174 patients with liver cirrhosis and in 7 out of 72 with acute leukaemia. Low values were most common in patients with myelomatosis (15 out of 158) and in pregnant women (13 out of 40). In none of the pregnant women was the level raised.

In most of the patients with deep venous thrombosis (118 out of 148) urokinase inhibitors were within normal limits; only 28 had elevated levels. Most patients with liver cirrhosis had normal levels (131 out of 174). Of the 29 women using low dosage gestagen contraceptives the values were normal in 21, slightly increased in 7 and somewhat decreased in only one.

Neither the α_2 -macroglobulin nor the α_1 -antiplasmin varied with the urokinase inhibitors. In renal disease the levels of both α_2 -macroglobulin and α_1 -antiplasmin

were normal (mean 100 % and 102 % respectively in chronic uraemia and 93 and 95 % in acute uraemia). In patients examined after renal transplantation the α_2 -macroglobulin levels were around the lower limit of normal (mean 77 %), while the α_1 -antiplasmin was normal (mean 103 %). In the pregnant women, in whom the level of urokinase inhibitor was near the lower normal limit (mean 79 %) both α_2 -macroglobulin and α_1 -antiplasmin were often increased (means 121 % and 157 % respectively).

V. Demonstration of low content of fibrinolytic inhibitors in individuals with high fibrinolytic capacity

The clinical material consisted of 4 apparently healthy individuals who had been shown by Jacobsen (1968) to have a high proteolytic capacity (HPC). The subjects included a father and his son. Sera from 24 apparently healthy persons served as controls in the gel filtration experiments.

The following determinations were made: plasminogen by all three methods, α_1 -antiplasmin, α_2 -macroglobulin, total antitrypsin activity (TAT) and the urokinase inhibitors by both the clot lysis method (Paraskevas et al 1962) and the caseinolytic method (Lauritsen 1968). Gel filtration of the sera from the HPC subjects and from the 24 controls was performed on Sephadex G.200.

In 2 of the HPC subjects, namely the son and father, the fibrinolytic components were normal except the urokinase inhibitors which were extremely low (0-20 %). In the other 2 subjects the urokinase inhibitors were also decreased, and these also had increased amounts of plasminogen.

Gel filtration on Sephadex G-200. After gel filtration on Sephadex G-200 of the control sera, the α_2 -macroglobulin was found in a peak in the high molecular weight protein zone. The α_1 -antiplasmin was obtained in two peaks, a small one in the high molecular weight zone and a large one in the albumin peak. Plasminogen produced a well-defined peak in the 2nd protein peak. The inhibitors of urokinase activation of plasminogen produced two peaks when determined with the clot lysis method. The first was wide and plump, partly overlapping the α_2 -macroglobulin peak, and the other was situated in front and in the anterior part of the albumin peak. When determined by the caseinolytic method, the first peak separated into two. The third peak had the same position as the second peak indicated by the clot method. After heating (30 min, 56°C) of normal sera only the first peak of the urokinase inhibitor then identical with the unchanged α_2 -macroglobulin peak

was still to be seen. In serum heated before gel filtration no or almost no urokinase inhibitors was demonstrable.

After gel filtration of sera from the HPC subjects, the first peak with activity inhibiting urokinase activation of plasminogen showed up normally but the second peak was missing in 3 of the 4 HPC subjects and very small in the fourth. The plasminogen peak was normal in 2 of the subjects and higher in the two with increased serum plasminogen. Both position and shape of the peak were normal.

Urokinase inhibitors were still not demonstrable after adsorption, with bentonite to remove plasminogen from serum and fractions obtained after gel filtration from the 2 subjects with high plasminogen. The gel filtration patterns of sera were the same before and after adsorption with bentonite.

V I. S t u d i e s o n f i b r i n o l y t i c i n h i b i t o r s d u r i n g p r e g - n a n c y

The clinical material consisted of 120 apparently healthy pregnant women, 40 in each trimester. Sera from 40 healthy age-matched women not using oral contraceptives served as controls.

The following determinations were made: α_2 -macroglobulin, α_1 -antiplasmin, urokinase inhibitors both with the clot lysis method (Paraskevas et al 1962) and caseinolytically (Lauritsen 1968), total antitrypsin activity (TAT) and plasminogen immunochemically (Ekelund et al 1970). Sera from 25 pregnant women in the 3rd trimester were also submitted to gel filtration on Sephadex G-200.

The α_2 -macroglobulin (Fig. 3) were increased most during the first two trimesters ($p < 0.001$). In the 3rd trimester there was a small decrease towards normal ($p < 0.05$).

The α_1 -antiplasmin activity (Fig. 3) rose progressively throughout pregnancy and was significantly elevated already in the 1st trimester ($p < 0.001$).

Total antitrypsin activity (TAT) was increased throughout pregnancy (Fig. 3).

Plasminogen (Fig. 3) was increased in all trimesters ($p < 0.001$).

Urokinase inhibitors did not differ significantly from normal in the first 2 trimesters. During the 3rd trimester a slight but significant decrease ($p < 0.001$) was found with the clot lysis method but not with the caseinolytic method (mean 0.045).

Gel filtration on Sephadex G-200 of sera from pregnant women in the 3rd trimester gave an almost normal pattern as for α_2 -macroglobulin. The first antiplasmin peak was also normal, while the second was abnormally wide and high. The pattern of urokinase inhibitors showed a normal first peak, while the second were in most patients decreased or absent. Total urokinase inhibitory activity in the two peaks did not, however, significantly differ from normal ($p > 0.05$).

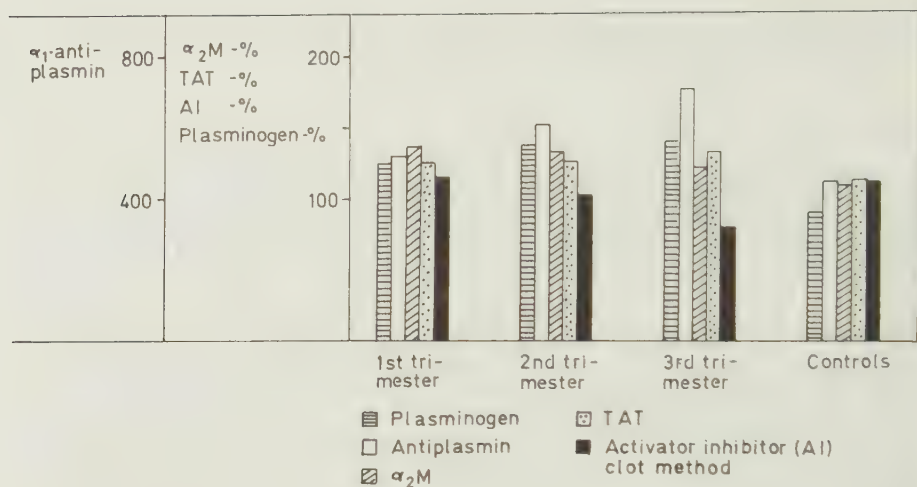


Fig. 3 Mean values of the different fibrinolytic inhibitors and plasminogen in 120 pregnant and 40 non-pregnant women (controls) α_2 M = α_2 -macroglobulin, TAT = total antitrypsin activity

VII. Bilateral occlusion of the retinal veins in a patient with inhibition of fibrinolysis

A 53 year-old man developed occlusion of the retinal veins on both sides within a period of two years. The following determinations were made: platelet adhesiveness in whole blood, coagulation time, one-stage prothrombin time, recalcification time of plasma, prothrombin + factor VII + factor X (Owren's P&P test), factor V, fibrinogen and thrombin time. The fibrinolytic system was assayed as following: spontaneous fibrinolytic activity of plasma and of redissolved euglobulin precipitate on fibrin plates, plasminogen, α_2 -macroglobulin, α_1 -antiplasmin, inhibitors of the urokinase activation of plasminogen and the fibrin/fibrinogen degradation products (FDP). Fibrinolytic response to venous occlusion of the arms and fibrinolytic activator in vessel walls were also determined. The patient's serum was gel filtered on Sephadex G-200.

All results including fibrinolytic activity of the vessel wall were within normal limits except those for plasminogen and urokinase inhibitors. The inhibitors were markedly increased (423-700 %) on 3 occasions; plasminogen was diminished (43-46 %).

After gel filtration of serum both fractions of urokinase inhibitors were increased, mostly in the second, low molecular weight peak. The patterns for α_2 -macroglobulin and α_1 -antiplasmin were normal.

VIII. Studies on an inhibitor of plasminogen activation in human serum

Sera from apparently healthy persons were used.

Purification procedure. 1) Haptoglobin was removed by passing serum through a column of Sepharose 4 B to which haemoglobin had been covalently coupled. All proteins except haptoglobin passed through the column and the haptoglobin content was afterwards 1 %.

2) Ion exchange chromatography of serum deprived of haptoglobin was done on DEAE-Sephadex with phosphate buffer (0.01 M, pH 8.6). No gradient was used. After dialysis against Tris buffer (0.07 M, pH 7.8 ionic strength 0.15), the different inhibitors (α_2 -macroglobulin, α_1 -antiplasmin and urokinase inhibitors) were assayed in the fractions (3 ml each). The activator inhibitory activity was obtained in 2 well separated peaks. The first was smaller and coincided with the α_2 -macroglobulin. The second, containing most of the activity, was obtained at effluent volume of 72 ml. This fraction also contained plasminogen, prothrombin, α_1 -antiplasmin, ceruloplasmin and some albumin. The fractions containing albumin were removed, after which the other fractions in the second peak were pooled and concentrated to about 3 ml against a membrane retaining all protein with a molecular weight of more than 25,000.

3) Gel filtration on Sephadex G-200 of the pooled fraction from step 2 was performed in Tris buffer (0.07 M, pH 7.8). Activator activity was in two peaks, the smaller in the high molecular weight zone (effluent volume of 42 ml; $K_d = 0.06$) and the larger in the low molecular weight region (effluent volume of 74 ml; $K_d = 0.19$). The large peak also contained plasminogen, ceruloplasmin, α_1 -antiplasmin and prothrombin. The fractions containing the α_1 -antiplasmin were removed and the other fractions were pooled and concentrated. Fractions obtained from 3 different Sephadex columns were pooled.

4) Preparative gel electrophoresis was performed in agarose gel (1 % 2 mm thick) of the concentrated activator inhibitor fraction from step 3 (originating from 9 ml serum). On application the sample was mixed with 3 % agarose solution. The electrophoresis was run at 200 V for 1/2 hour in barbital buffer (0.075 M, pH 8.6

containing calcium lactate). The gel was then cut into 0.5 slices, which were placed in tubes and frozen at -70°C for 30 minutes. Afterwards they were thawed at room temperature and centrifuged. The supernatant was removed and tested for activator inhibitor, plasminogen, α_2 -macroglobulin, α_1 -antiplasmin, ceruloplasmin, total antitrypsin, antithrombin III and prothrombin activity. The activator inhibitory activity was found in one band in the α_2 -region. The eluate of this band yielded one band on agarose electrophoresis run for 3 hours in a barbital buffer (0.075 M, pH 8.6 containing calcium lactate). No plasminogen, α_1 -antiplasmin, total antitrypsin and no antithrombin III activity could be demonstrated. The content of activator inhibitor varied between 20 and 30 % of that of normal serum, which represents a recovery of about 1 %. The activity was 30 % of that in normal serum per mg protein.

Experiments to establish the identity of the activator inhibitor. 1) Plasminogen, α_1 -antiplasmin, total antitrypsin activity and antithrombin III could not be demonstrated in the eluate from step 4.

2) Agarose gel electrophoresis. The eluate of the α_2 -band from step 4, which contained all activator inhibitor activity, yielded one band on agarose electrophoresis.

3) Analytical disc electrophoresis in polyacrylamide produced three bands, one distinct and two vague, indicating contamination with some other proteins in spite of the fact that the fraction appeared homogeneous on the basis of agarose electrophoresis.

4) Immunological properties. Antiserum against the inhibitor fractions produced, without any absorption procedure, 3 distinct peaks in crossed immunoelectrophoresis (Ganrot 1972). By immunological identification (Kröll 1968), one of these peaks proved identical with ceruloplasmin and another with prothrombin. After absorption of the antiserum by ceruloplasmin and human prothrombin there was only one peak. No precipitation lines were found against α_2 -macroglobulin or inter- α -inhibitor antiserum.

5) Molecular weight determination by the gel filtration method (Whitaker 1963) gave a value of 75,000 for the activator inhibitor.

6) Functional tests of the inhibitor fraction showed

that it inhibited also the activation of plasminogen by streptokinase.

7) Thermostability. Freezing and thawing of the preparation reduced the activity markedly which was abolished by storage at -20°C for two months. After storage for 4 hours at room temperature the activity decreased almost to zero as well as after dialysis at $+4^{\circ}\text{C}$ for more than 2 hours. The preparation also lost its activity when heated to 56°C for 30 min. Further experiments to differentiate the inhibitors by their thermostability were carried out by assaying the α_2 -macroglobulin, the α_1 -antiplasmin and the activator inhibitor in 10 normal human sera before and after heating at 56°C for 30 min (Table IV). The activator inhibitor in the heated samples was completely destroyed, the α_1 -antiplasmin, as measured with a caseinolytic method, decreased from 45 to 20 CTA units/ml and the α_2 -macroglobulin was thermostable.

8) Stability in acid milieu (Table IV). After acidification of 20 normal sera to pH 2.0 for 15 min, all inhibitors were almost completely inactivated.

9) Treatment with methylamine (Table IV). In some other experiments, in which methylamine was added to a final concentration of 0.2 M to 13 human normal sera, the substance was found to abolish the α_2 -macroglobulin activity, decrease activator inhibitor activity from 94 % to 37 %, but had no effect on α_1 -antiplasmin activity.

Table IV

The reaction of the different antiplasmins and the activator inhibitor to heating (56°C, 30 min), acidification (pH 2.0) and treatment with methylamine (0.25 M final conc) (+ = inactivation)

	Inactivation by heating 56°C	0.25 M methyl- amine	Acidifica- tion (pH 2.0)
α_2 -macro- globulin	-	+	+
α_1 -anti- plasmin	+	-	+
Inter- α - inhibitor (protein π)	-	-	-
Activator inhibitor	+	(+)	+

IX. Pattern of the activator inhibitor in carcinoma and renal diseases

Sera from 20 patients with malignant diseases (carcinoma of the intestines, the pancreas, the breast, the prostate or Hodgkin's disease), 12 with chronic renal insufficiency (glomerulonephritis, pyelonephritis or polycystic renal disease) and 10 with acute renal insufficiency due to tubular necrosis or acute glomerulonephritis were subjected to gel filtration on Sephadex G-200.

Activator inhibitory activity obtained after gel filtration was determined with the clot lysis method (Paraskevas et al 1962). In patients with malignant diseases and chronic renal failure the activator inhibitor was also determined caseinolytically (Lauritsen 1968, Hedner et al 1970; V). α_1 -macroglobulin was determined with the esterolytic method of Ganrot (1966).

Malignant diseases (20 patients). The activator inhibitory activity present in the high molecular fraction ($K_d = 0.03$) did not differ from normal ($p > 0.05$), whereas the activity in the low molecular weight fraction ($K_d = 0.21$) was significantly higher ($p < 0.001$) than normal (Table V). With the caseinolytic method the inhibitory activity in the two high molecular weight fractions did not differ from normal ($p > 0.05$). The inhibitory activity in the low molecular weight fraction was increased ($0.02 < p < 0.05$).

Chronic uraemia (12 patients). After gel filtration (Table V) the inhibitory activity with the clot method was significantly higher than normal in both fractions ($p < 0.01$ for the high molecular weight fraction; $K_d = 0.02$ and $p < 0.001$ for the low molecular weight fraction; $K_d = 0.19$). When determined caseinolytically the inhibitory activity found in the different fractions did not differ significantly from normal.

Acute uraemia (10 patients). After gel filtration (Table V) of the sera the activator inhibitory activity determined with the clot method was significantly higher in both the high molecular weight fraction ($p < 0.01$, $K_d = 0.02$) and the low molecular weight fraction ($p < 0.001$, $K_d = 0.19$).

Three of the patients, all with tubular necrosis, were examined 2-3 times in the course of the disease. When the patients improved the inhibitor activity in the serum determined with the clot method decreased; in one of the patients, to normal. The low molecular weight fraction obtained after gel filtration decreased with improvement of the patient.

Normal serum to which δ -aminolevulinic acid had been added was subjected to gel filtration on Sephadex G-200 (Table V). The inhibitory activity determined with the clot method was obtained in two fractions, one in the high molecular weight zone ($K_d = 0.04$); the other in the low molecular weight zone ($K_d = 0.19$). Inhibitory activity was higher than normal in both the high molecular weight (mean of 5 determinations 179 %; normal 54 %, $N = 24$) and in the low molecular weight fraction (mean of 5 determinations 222 %; normal 22 %, $N = 24$).

There was no covariation in any of the patient groups between the serum values of activator inhibitory activity and α_2 -macroglobulin.

Table V

Mean values of the total inhibitory activity determined with the clot lysis method in the two fractions obtained after gel filtration on Sephadex G-200 of serum from patients of different categories, controls and control sera to which δ -amino-levulinic acid has been added

Diagnoses no. of cases	Tot. inhibitory activity in the	
	high mol.wt frac- tion - %	low mol.wt frac- tion - %
Malignant diseases (20)	46	51
Chronic uraemia (12)	103	87
Acute uraemia (10)	70	76
Normal sera + δ -aminolevulinic acid (5)	179	222
Controls (24)	54	22

Further immunochemical experiments

Antiserum against activator inhibitor prepared in rabbits as described in VIII and absorbed with ceruloplasmin and prothrombin was used for determination of the activator inhibitor in 20 apparently healthy persons and in 10 patients with chronic renal failure (glomerulonephritis or pyelonephritis). The rocket method of Laurell (1966) was used and a pooled serum from 25 apparently healthy persons served as standard. Parallel determination were made of the inhibitory activity with the clot method of Paraskevas et al (1962). In the uraemic patients the inhibitor activity ranged between 81 and 159 % (mean 116 %) and the immunochemical determinations gave values between 43 and 113 % (mean 66 %). As seen in Table VI, the values obtained with the two methods did not vary together. The immunochemical values were lower than the activity in all but one of the patients. Serum from some of the patients was also subjected to gel filtration and the two fractions with activator inhibitor activity were concentrated separately. The fractions were then tested against the antiserum with the rocket method. There was no covariation with the activator inhibitory activity in serum. In the 20 controls the mean value with the clot lysis method was 104 % (range 70-142 %) and with the immunochemical one 105 % (range 84-140 %).

Normal serum was subjected to gel filtration on Sephadex G-200. All fractions containing activator inhibitory activity were tested against the antiserum with the rocket method. Only the fractions in the low molecular weight zone gave precipitates against the antiserum.

The inhibitor fraction obtained according to the procedure described in VIII, was subjected to crossed immunoelectrophoresis as described by Ganrot (1972). When diluted to 5 % as determined with the clot lysis method it gave an immunochemically amount reflected by an area of 117 mm², while normal human serum diluted to the same activity (clot lysis method) gave an area of 57 mm² (Fig. 4).

Table VI

The activator inhibitor levels obtained with the clot lysis method of Paraskevas et al (1962) and immunochemically in serum from 10 uraemic patients

Case no	Activator inhibitor activity	
	clot method - %	imm.chem. method - %
1	114	50
2	117	50
3	101	67
4	119	97
5	82	43
6	81	113
7	129	45
8	159	50
9	128	86
10	125	57
Mean	115.5	65.8
Range	81-159	45-113
Controls (20)		
Mean	104.2	105.3
Range	70-142	84-156



Fig. 4 Crossed immunoelectrophoresis of the activator inhibitor fraction prepared as described in VIII (to the left) and of normal serum (to the right). Both had an activity of 5 % according to the clot lysis method

DISCUSSION

Inhibition of activation of plasminogen in serum has been reported by some (Müllertz 1954a, Nilsson et al 1961b, Paraskevas et al 1962, Brakman et al 1966, Lauritsen 1968, Bennett 1967, 1970, den Ottolander et al 1969a), but questioned by others (Dudok de Wit 1964, Jacobsen 1964). Nilsson et al (1961b) described a patient with recurrent deep venous thrombosis and complete inhibition of the fibrinolytic system due to a high content of inhibitors of the plasminogen activation. That patient had a normal level of α_1 -antiplasmin. In VII a similar patient was described. The patient who had had bilateral retinal thrombosis, had markedly raised levels of activator inhibitor, while both α_2 -macroglobulin and α_1 -antiplasmin were repeatedly found to be within normal limits. No other factors were found which are known to predispose to venous thrombosis, such as decreased content of plasminogen activator in the vessel walls and/or defective release of this activator; this occurs in 73 % of patients with thrombotic disease (Isacson & Nilsson 1972). Only 19 % of patients with recurrent deep venous thrombosis had raised levels of the activator inhibitor (IV), indicating that an increase of this inhibitor is a rare but significant cause of thrombotic disease. A selective increase of activator inhibitor was also found by Brakman et al (1966) in a group of patients with severe thrombotic disease. An increase of the inhibitory activity against the activation of plasminogen occurs after trauma (Bergentz & Nilsson 1961, Rammer 1972). This inhibition is assumed to play a role in the development of post-traumatic and postoperative thrombosis (Bergentz & Nilsson 1961) and in posttraumatic deposition of fibrin in the lungs (Bagge et al 1973).

Very little is known about the nature of the activator inhibitor in human plasma. Most methods for demonstrating the inhibitors make use of clot lysis in which it is difficult to differentiate between activator inhibition and antiplasmin effect (Nilsson et al 1961b, Paraskevas et al 1962, Bennett 1967, 1970, den Ottolander et al 1969a). Bennett (1967), however, pointed out the lack of correlation between inhibition in his clot lysis system and antiplasmin activity determined caseinolytically. Further studies have also shown, that urokinase inhibitors measured by clot lysis (Paraskevas et al 1962) do not vary with the α_2 -macroglobulin or antiplasmin (IV, VI, VII, IX). This indicates that inhibitory acti-

vity measured with this clot lysis method is mainly due to specific inhibition of the activation of plasminogen and not due to α_2 -macroglobulin or α_1 -antiplasmin. The immunochemical results reported in VIII lend further support to the assumption that human serum contains a specific inhibitor of plasminogen activation and that it is not identical with α_2 -macroglobulin, antiplasmin or the inter- α -inhibitor described by Heide et al (1965). So did the studies of its physical properties (VIII), according to which the activator inhibitor differed (Table IV) from α_2 -macroglobulin, α_1 -antiplasmin and inter- α -inhibitor (Norman & Hill 1958, Heide et al 1965, Rimon et al 1966, Schwick et al 1966, Rowley & Oette 1973).

A method which differentiates between the activator inhibitor effect and the antiplasmin effect has been elaborated by Lauritsen (1968), who used a caseinolytic system. This method was shown (V, VI, IX) to determine essentially the same protein as the clot lysis method of Paraskevas et al (1962). But in IX it was demonstrated that patients with renal diseases often had high levels of urokinase inhibitors as measured with the clot lysis method, while the levels measured caseinolytically were within the normal range, at least in patients with chronic uraemia; this was confirmed by gel filtration. This may suggest that the caseinolytical method is more specific for measuring the protein with activator inhibiting effect, whereas unspecific inhibitory activity is included in the clot lysis test.

It is known that the fibrinolytic activity in plasma from uraemic patients is low (Edward et al 1964, Larsson et al 1971a,b); this has been tentatively ascribed to an increased activator inhibitory activity (Isacson & Nilsson 1969, Larsson et al 1971a,b). This is supported by the finding of high levels of activator inhibitors in patients with chronic and acute uraemia (IV, IX). After gel filtration of serum from uraemic patients both the high and low molecular weight fractions were increased (IX), whereas only the low molecular weight fraction was increased in patients with malignant diseases (IX). Furthermore, the uraemic pattern did not differ from normal when determined caseinolytically (IX). This suggests a different mechanism of activator inhibition in uraemic patients. Plasmas of uraemic patients contain increased concentrations of certain amino acids, especially glycine, arginine and proline (Dunn et al 1956, Salisbury et al 1957, Frimpter et al 1961, McGale et al 1972). Müllertz (1954b) found that lysine, ornithine, and to

a lesser extent glycine, inhibit plasminogen activation. Sjoerdsma & Nilsson (1960) demonstrated that aliphatic amino acids with the amino groups in a terminal position, including δ -aminolevulinic acid and ornithine, inhibit the activation of plasminogen. During the metabolism of glycine both ornithine (during the synthesis of creatinine) and δ -aminolevulinic acid (during the synthesis of porphyrins) are formed. After gel filtration of normal serum to which δ -aminolevulinic acid had been added, inhibition of plasminogen activation appeared in two fractions which probably corresponded to those obtained after gel filtration of normal serum or serum from uraemic patients as shown by the K_d -values (IX). These observations suggest that the amino acid links to or activates a protein in the plasma. It thus seems possible that the inhibitory activity of plasminogen activation in serum from patients with renal failure may depend partly on retained amino acids. The low levels of the activator inhibitor protein, as measured immunochemically also speaks in favour of the occurrence of an unspecific inhibition in these patients.

The pattern of the activator inhibitor obtained after gel filtration of normal human serum on Sephadex G-200 (V) as well as the molecular weight of the main (low molecular weight) fraction (75,000) (VIII) suggest that it is identical with the activator inhibitor described by Aoki & v.Kaulla (1971b). Their inhibitor was active against human vascular plasminogen activator instead of urokinase. This suggests that the activator inhibitor occurring in human serum can inhibit the activation of plasminogen by different activators, which may indicate that the inhibitor acts on the plasminogen molecule, as proposed by Lauritsen (1970), and not on the activator. The main fraction, that is the one with the lower molecular weight, was lacking in some patients with a low serum activator inhibitor activity (V), thus suggesting that it is, above all, this fraction that is responsible for the inhibitor activity measured in serum. This was sustained by the finding of an increase in this fraction in patients with carcinoma (IX), in a patient with recurrent retinal thrombosis (VII) and the absence of the low molecular weight fraction in some pregnant women with a low serum level of the activator inhibitor (VI). The high molecular weight fraction contains, however, most probable the low molecular weight inhibitor in aggregated form as indicated by the fact that this fraction disappears and the low molecular weight fraction increases when serum is fractionated

with glycine (0.5 M) in the buffer (unpublished data). The shape of the immunoprecipitate obtained on crossed immunoelectrophoresis of normal human serum also suggest a heterogeneity of the protein (a broad not very distinct precipitate). Furthermore the heat lability found in both the low molecular weight fraction and the shoulder of the curve produced by the high molecular weight fraction (V) sustain this hypothesis.

The activator inhibitors found by Nilsson et al (1961b) in serum proved to be α_2 -globulin, thermolabile, non-dialysable and not adsorbed to BaSO_4 . They were not lipoproteins. Apart from this very little is known about the activator inhibitors in human serum, probably because of their extreme lability (VIII, V). A human serum activator inhibitor probably identical with that described by Nilsson et al (1961b) was studied in VIII. By means of a specific adsorption technique to eliminate haptoglobin from the serum, it was possible to prepare an inhibitor fraction contaminated with only small amounts of prothrombin and ceruloplasmin. The remarkably low recovery when determined with the clot lysis test obtained after the isolation procedure described (VIII) most probably depend on the lability of the inhibitor. This is strengthened by the fact that the immunologically obtained inhibitor amount of the preparation was about twice as high as that of normal human serum although the biological activity was identical. The inhibitor may be a very low molecular weight substance which aggregates or links to other proteins and easily dissociates again and loses its activity.

Urokinase inhibitors have been isolated from placenta and purified (Kawano et al 1970, Uszynski & Abildgaard 1971). But these inhibitors differ in many respects from those found in human serum (VIII). Thus, they did not inhibit the streptokinase activation of plasminogen. Further on, the inhibition described by Kawano et al (1970) proved to be acid stable and did not lose its activity when stored at $+2^\circ\text{C}$ for 12 months. The molecular weight of the placenta, inhibitors were found to be 43,000 and 100,000, respectively. It would thus appear that there may exist a variety of inhibitors of plasminogen activation, as also suggested by Brakman et al (1966).

Summing up, the following observations were made in the studies reported above.

- 1) Inhibitors against the plasminogen activation by uro-

kinase not identical with the antiplasmin or α_2 -macroglobulin occur in human serum (VIII). This inhibitor was found to vary markedly in different clinical conditions independently of the antiplasmin or the α_2 -macroglobulin (IV, V, VI, VII, IX).

2) After gel filtration of human serum on Sephadex G-200 (V) the activator inhibitor was obtained in two fractions, a minor one with a high molecular weight and a major one with a low molecular weight. The low molecular weight fraction was also shown to reflect the serum values of the urokinase inhibitor in patients with low activator inhibitor levels (V, VI), in patients with carcinoma (IX) and in a patient with recurrent venous thrombosis (VII). It is suggested that the high molecular weight fraction contain the activator inhibitor in aggregated form or linked to another protein.

3) A method for partial purification of the main fraction of the activator inhibitor obtained after gel filtration (mol.wt 75,000) was devised and an anti-serum against this inhibitor was raised in rabbits (VIII). Although traces of prothrombin and ceruloplasmin contaminated the fraction it was found to be completely separated from the α_1 -antiplasmin as well as the α_2 -macroglobulin and inter- α -inhibitor (VIII).

4) The elevation of the urokinase inhibitors found in serum in patients with renal disorders was reflected by an increase in both the high molecular weight and the low molecular weight fraction obtained on gel filtration on Sephadex G-200 when measured with the clot lysis method of Paraskevas et al (1962). No such changes were found when the caseinolytic method of Lauritsen (1968) was used, thus indicating an unspecific inhibitor effect in uraemic patients (IX). It was shown that the same pattern occurred after gel filtration of human serum to which certain amino acids had been added showed the same pattern thus indicating the possibility that amino acids accumulated in plasma in uraemia may bind to some plasma protein and exert an inhibitory effect on the activation of plasminogen (IX).

REFERENCES

- Abiko, Y., Iwamolo, M. & Tomikana, M.: Plasminogen-plasmin system. V. A stoichiometric equilibrium complex of plasminogen and a synthetic inhibitor. *Biochim Biophys Acta* 185:424, 1969.
- Ablondi, F.B., Hagan, J.J., Philips, M. & de Renzo, E.C.: Inhibition of plasmin, trypsin and the streptokinase-activated fibrinolytic system by ϵ -aminocaproic acid. *Arch Biochem Biophys* 82:153, 1959.
- Adelson, E.: Fibrinogen metabolism. In *Fibrinogen*, ed. Laki, K. Marcel Dekker, New York 1968, p 225.
- Albrechtsen, O.K.: The fibrinolytic activity of human tissues. *Brit J Haemat.* 3:284, 1957.
- Albrechtsen, O.K.: The fibrinolytic agents in saline extracts of human tissues. *Scand J clin Lab Invest* 10:91, 1958.
- Alkjaersig, N., Fletcher, A.P. & Sherry, S.: The activation of human plasminogen. I. Spontaneous activation in glycerol. *J Biol Chem* 233:81, 1958a.
- Alkjaersig, N., Fletcher, A.P. & Sherry, S.: The activation of human plasminogen. II. A kinetic study of activation with trypsin, urokinase, and streptokinase. *J Biol Chem* 233:86, 1958b.
- Alkjaersig, N., Fletcher, A.P. & Sherry, S.: ϵ -aminocaproic acid: an inhibitor of plasminogen activation. *J Biol Chem* 234:832, 1959a.
- Alkjaersig, N., Fletcher, A.P. & Sherry, S.: The mechanism of clot dissolution by plasmin. *J clin Invest* 38:1086, 1959b.
- Alkjaersig, N., Fletcher, A.P. & Sherry, S.: Pathogenesis of the coagulation defect developing during pathological plasma proteolytic ("fibrinolytic") states. II. The significance, mechanism and consequences of defective fibrin polymerization. *J clin Invest* 41:917, 1962.
- Allington, M.J.: Detection of fibrin(ogen) degradation products by a latex clumping method. *Scand J Haemat Suppl* 13:115, 1971.
- Ambrus, C.M., Ambrus, J.L., Niswander, K.R., Weintraub, D.H., Bross, I.D.J. & Lassman, H.B.: Changes in fibrin-stabilizing factor levels in relation to maternal haemorrhage and neonatal disease. *Pediat Res* 4:82, 1970.
- Amery, A., Vermylen, J., de Vreker, R.A., Vermylen, C. & Verstraete, M.: Enhancing blood fibrinolytic activity by a niacin compound. II. Activation and non-activation. *Amer J med Sci* 249:66, 1965.

- Andersson, L.: Treatment of so-called essential haematuria with fibrinolytic inhibitor (epsilon-amino-caproic acid). *Acta chir scand* 124:355, 1962.
- Andersson, L., Nilsson, I.M. & Hedner, U.: Experimental and clinical studies on the antifibrinolytic and presumed antithrombotic effect of a kallikrein inhibitor (Trasylol). *Scand J Urol Nephrol* 1:11, 1967.
- Andersson, L., Nilsson, I.M., Nilsson, J.-E., Hedner, U., Granstrand, B. & Melander, B.: Experimental and clinical studies on AMCA, the antifibrinolytically active isomer of p-aminomethyl cyclohexane carboxylic acid. *Scand J Haemat* 2:230, 1965.
- Andersson, L., Nilsson, I.M., Liedberg, G., Nilsson, L., Rybo, G., Eriksson, O., Granstrand, B. & Melander, B.: Antifibrinolytica. Vergleichende Untersuchungen von trans-4-(Aminomethyl)-cyclo-hexan-carbonsäure, Aminocaprinsäure und p-Aminomethylbenzoesäure. *Arzneimittelforsch (Drug Res)* 21:424, 1971.
- Andersson, L., Nilsson, I.M. & Olow, B.: Fibrinolytic activity in man during surgery. *Thromb Diath haemorrh* 7:391, 1962.
- Ansari, A. & Silvis, S.E.: Plasma fibrinogen in health and disease. Its application in the differential diagnosis of an enlarged and irregular liver. *Amer J Gastroent* 57:13, 1972.
- Antoine, B., Neveu, Th. & Ward, P.D.: Fibrinuria during renal transplantation. *Transplantation* 8:98, 1969.
- Aoki, N. & v.Kaulla, K.N.: Dissimilarity of human vascular plasminogen activator and human urokinase. *J Lab clin Med* 78:354, 1971a.
- Aoki, N. & v.Kaulla, K.N.: Human serum plasminogen anti-activator: its distinction from antiplasmin. *Amer J Physiol* 220:1137, 1971b.
- Arieff, A.I. & Pinggera, W.F.: Rapidly progressive glomerulonephritis treated with anticoagulants. *Arch intern Med* 129:77, 1972.
- Arneson, H. & Fagerhol, M.K.: α_2 -macroglobulin, α_1 -antitrypsin, and antithrombin III in plasma and serum during fibrinolytic therapy with urokinase. *Scand J clin Lab Invest* 29:259, 1972.
- Arneson, H. & Godal, H.Chr.: Studies on the thrombin clotting time. I. The influence of fibrinogen degradation products. *Scand J Haemat* 10:232, 1973.
- Astrup, T.: A proteolytic inhibitor in lung tissue. 18th Intern. Physiol. Congr., Copenhagen. Abstracts, 81, 1950.
- Astrup, T.: Fibrinokinase. *Acta physiol scand* 24:267, 1951.

- Astrup, T.: Tissue activators of plasminogen. *Fed Proc* 25:42, 1966.
- Astrup, T. & Albrechtsen, O.K.: Estimation of the plasminogen activator and the trypsin inhibitor in animal and human tissues. *Scand J clin Lab Invest* 9:233, 1957.
- Astrup, T. & Permin, P.M.: Fibrinolysis in the animal organism. *Nature* 159:681, 1947.
- Astrup, T., Rasmussen, J., Amery, A. & Poulsen, H.E.: Fibrinolytic activity of cirrhotic liver. *Nature* 185:619, 1960.
- Astrup, T. & Sterndorff, I.: The plasminogen activator in animal tissue. *Acta physiol scand* 36:250, 1956.
- Auerswald, W., Binder, B. & Doleschel, W.: "Angiokinase" - molecular weights of proteins representing a perivascular plasminogen activator. *Thromb Diath haemorrh* 26:411, 1971.
- Auerswald, W., Binder, B. & Doleschel, W.: Angiokinase: Comparison of the concentration dependence of its plasminogen activator activity with that of urokinase. III Intern. Congr. Soc. Thromb. Haemostasis, Washington, D.C., Aug 22-26, 1972.
- Augener, W.: Immunanalyse von Glykoproteinen. In Peeters' Proc. XIIth Colloquium of Protides of the Biological Fluids, Brügge 1964 (Elsevier, Amsterdam) p 363, 1965.
- Axén, R., Porath, J. & Ernback, S.: Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* 214:1302, 1967.
- Bacani, R.A., Velasquez, F., Kanter, A., Pirani, C.L. & Pollak, V.E.: Rapidly progressive (nonstreptococcal) glomerulonephritis. *Ann intern Med* 69:463, 1968.
- Bachmann, F., Fletcher, A.P., Alkjaersig, N. & Sherry, S.: Partial purification and properties of the plasminogen activator from pig heart. *Biochemistry* 3:1578, 1964.
- Bagge, L., Busch, C., Rammer, L. & Saldeen, T.: Delayed fibrin elimination from the lungs of burned rats with endogenous inhibition of the fibrinolytic system. *Thrombosis Research* 2:365, 1973.
- Bailey, K. & Bettelheim, F.R.: The nature of the fibrinogen-thrombin reaction. *Brit med Bull* 11:50, 1955.
- Bailey, K., Bettelheim, F.R., Lorand, L. & Middlebrook, W.R.: Action of thrombin in the clotting of fibrinogen. *Nature* 167:233, 1951.
- Ball, A.P., Hill, R.L. & McKee, P.A.: The number of cross-link sites per subunit in fully crosslinked fibrin. III Congr. Intern. Soc. Thromb. Haemostasis, Washington, D.C. Aug 22-26, 1972.

- Barlow, G.H., Summaria, L. & Robbins, K.C.: Molecular weight studies on human plasminogen at the microgram level. *J Biol Chem* 244:1138, 1969.
- Barnhart, M. & Riddle, J.M.: Cellular localization of profibrinolysin (plasminogen). *Blood* 21:306, 1963.
- Beard, E.L., Busuttil, R.W. & Gottshalk, S.K.: Stress induced release of plasminogen activator from lysosomes. *Thromb Diath haemorrh* 21:20, 1969.
- Beard, E.L. & Hampton, J.K.: Effect of trauma on rat serum proteolytic activity. *Amer J Physiol* 204:405, 1963.
- Bennett, N.B.: A method for the quantitative assay of inhibitor of plasminogen activation in human serum. *Thromb Diath haemorrh* 17:12, 1967.
- Bennett, N.B.: Further studies on an inhibitor of plasminogen activation in human serum. *Thromb Diath haemorrh* 23:553, 1970.
- Bennett, N.B., Ogston, C.M. & Ogston, D.: Studies on the blood fibrinolytic enzyme system following acute myocardial infarction. *Clin Sci* 32:27, 1967.
- Bennett, N.M., Bennett, D., Holland, N.H. & Luke, R.G.: Serum fibrin degradation products in the diagnosis of transplantation rejection. *Transplantation* 14:311, 1972.
- Bergentz, S.-E. & Nilsson, I.M.: Effect of trauma on coagulation and fibrinolysis in dogs. *Acta chir scand* 122:21, 1961.
- Bergstein, J.M. & Michael, A.F.: Cortical fibrinolytic activity in normal and diseased human kidneys. *J Lab clin Med* 79:701, 1972.
- Bergström, K.: A preparative method for the partial purification of human urokinase. *Arkiv Kemi* 21:535, 1963.
- Bergström, K., Tse, A.O. & Johnson, A.J.: Large-scale production of intermediate-purity soluble human plasminogen. *Thromb Diath haemorrh* 25:105, 1971.
- Bernik, M.B. & Kwaan, H.C.: Origin of fibrinolytic activity in cultures of the human kidney. *J Lab clin Med* 70:650, 1967.
- Biggs, R., Macfarlane, R.G. & Pilling, J.: Observations on fibrinolysis. Experimental activity produced by exercise or adrenaline. *Lancet* 1:402, 1947.
- Björkman, S.E.: A new method for enumeration of platelets. *Acta Haemat* 22:377, 1959.
- Blix, S.: The proactivator of the fibrinolytic system in human plasma. The quantitative determination and its clinical application. *Acta med scand* 171:83, 1962.

- Blix, S.: The quantitative determination of fibrinolytic inhibitors in plasma or serum by means of the fibrin plate. *Scand J clin Lab Invest* 16:403, 1964.
- Blombäck, B.: Subunit structure of fibrinogen. *Scand J Haemat Suppl* 13:71, 1971.
- Blombäck, B. & Blombäck, M.: Purification of human and bovine fibrinogen. *Arkiv Kemi* 10:415, 1956.
- Blombäck, B., Blombäck, M., Henschen, A., Hessel, B., Iwanaga, S. & Woods, K.R.: N-terminal disulphide knot of human fibrinogen. *Nature* 218:130, 1968.
- Blombäck, B., Hessel, B., Iwanaga, S., Reuterby, J. & Blombäck, M.: Primary structure of human fibrinogen and fibrin. I. Cleavage of fibrinogen with cyanogen bromide. Isolation and characterization of NH₂-terminal fragments of the α ("A") chain. *J biol Chem* 247:1496, 1972.
- Blombäck, B. & Yamashina, I.: On the N-terminal amino acids in fibrinogen and fibrin. *Arkiv Kemi* 12:299, 1958.
- Blombäck, M.: Molecular structure of fibrinogen. III Intern. Congr. Thromb. Haemostasis, Washington, Aug 22-26, 1972.
- Bohn, H., Becker, W. & Trobisch, H.: Die molekulare Struktur der fibrinstabilisierenden Faktoren des Menschen. II. Vergleichende immunologische Untersuchungen von Faktor XIII-Mangelplasmen und Normalplasma. *Blut* 26:303, 1973.
- Bohn, V.H., Haupt, H. & Kranz, Th.: Die molekulare Struktur der fibrinstabilisierenden Faktoren des Menschen. I. *Blut* 25:235, 1972.
- Bohn, H. & Schwick, H.G.: Isolierung und Charakterisierung eines fibrinstabilisierenden Faktors aus menschlichen Plazenten. *Arzneimittelforsch* 21:1432, 1971.
- Bonnar, J., McNicol, G.P. & Douglas, A.S.: Fibrinolytic enzyme system and pregnancy. *Brit med J* 3:387, 1969.
- Boomgaard, J., Vreeken, J., Bleyenbergh, A. & Deggeller, K.: Studies on urokinase. Some physiological considerations concerning normal urokinase excretion. *Clin Chim Acta* 13:484, 1969.
- Boyer, M.H., Shainoff, J.R. & Ratnoff, O.D.: Acceleration of fibrin polymerization by calcium ions. *Blood* 39:382, 1972.
- Brakman, P.: Bovine fibrinogen without detectable plasminogen. *Analyt Biochem* 11:149, 1965.
- Brakman, P. & Astrup, T.: Selective inhibition in human pregnancy blood of urokinase induced fibrinolysis. *Scand J clin Lab Invest* 15:603, 1963.

- Brakman, P., Mohler, E.R. & Astrup, T.: A group of patients with impaired plasma fibrinolytic system and selective inhibition of tissue activator-induced fibrinolysis. *Scand J Haemat* 3:389, 1966.
- Braun, W.E. & Merrill, J.P.: Urine fibrinogen fragments in human renal allografts. *New Engl J Med* 278:1366, 1968.
- Brian, M.C., Baker, L.R.J., McBride, J.A. & Rubenberg, M.: Heparin therapy in the haemolytic-uraemic syndrome. *Quart J Med* 36:608, 1967.
- Briggs, J.D., Prentice, C.R.M., Hutton, M.M., Kennedy, A.C. & McNicol, G.P.: Serum and urine fibrinogen-fibrin-related antigen (F.R.-antigen) levels in renal disease. *Brit med J* 4:82, 1972.
- Britton, B.J., Wood, W.G., Irving, M.H. & Hawkey, Ch.: The changes in fibrinolysis and blood catecholamines associated with muscular exercise and the effect of various beta adrenergic antagonists. IVth Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Brockway, W.J. & Castellino, F.J.: The mechanism of the inhibition of plasmin activity by ϵ -aminocaproic acid. *J biol Chem* 246:4641, 1971.
- Brockway, W.J. & Castellino, F.J.: Measurement of the binding of antifibrinolytic amino acids to various plasminogens. *Arch Biochem Biophys* 151:194, 1972.
- Brown, R.K., Baker, W.H., Peterkofsky, A. & Kauffman, D.L.: Crystallization and properties of a glycoprotein from human plasma. *J amer chem Soc* 76:4244, 1954.
- Budzynski, A.Z., Stahl, M., Kopec, M., Latallo, Z.S., Wegrzynowicz, Z. & Kowalski, E.: High molecular weight products of the late stage of fibrinogen proteolysis by plasmin and their structural relation to the fibrinogen molecule. *Biochim Biophys Acta* 147:313, 1967.
- Bundy, H.F. & Mehl, J.W.: Trypsin inhibitor of human serum. II. Isolation of the α_1 -inhibitor and its partial characterization. *J biol Chem* 234:1124, 1959.
- Burges, R.A., Brammer, K.W. & Coombes, J.D.: Molecular weight of urokinase. *Nature* 208:894, 1965.
- Cade, R., Spooner, G., Schlein, E., Pickering, M., deQuesada, A., Holcomb, A., Juncos, L., Richard, G., Shires, D., Levin, D., Hackett, R., Free, J., Hunt, R. & Fregly, M.: Comparison of Azathioprine, Prednisone, and heparin alone or combined in treating lupus nephritis. *Nephron* 10:37, 1973.
- Campra, J., Ashcavai, M., Peters, R.L. & Redeker, A.: Serum antitrypsin levels in acute hepatic necrosis. *Brit med J* 2:616, 1973.

- Carlsson, S., Hedner, U., Nilsson, I.M., Bergentz, S.-E. & Ljungqvist, U.: Kidney transplantation and fibrinolytic split products in serum and urine. Transplantation 10:366, 1970.
- Cash, J.D. & Allan, A.G.E.: The fibrinolytic response to moderate exercise and intravenous adrenaline in the same subjects. Brit J Haemat 13:376, 1967.
- Cash, J.D. & Clarkson, A.R.: Serum and urinary fibrin/fibrinogen degradation products in renal disease. Scand J Haemat Suppl 13:331, 1971.
- Cash, J.D., Woodfield, D.G. & Allan, A.G.E.: Adrenergic mechanisms in the systemic plasminogen activator response to adrenaline in man. Brit J Haemat 18:487, 1970.
- Catanzaro, A., Strathern, J. & Edgington, T.S.: The subunit structure and in vivo behavior of fibrinogen fragment D. Fed Proc 30:339, 1971.
- Celander, D.R. & Guest, M.M.: The biochemistry and physiology of urokinase. Amer J Cardiol 6:409, 1960.
- Chakrabarti, R., Birks, P.M. & Fearnley, G.R.: Origin of blood fibrinolytic activity from veins and its bearing on the fate of venous thrombi. Lancet 1:1288, 1963.
- Chakrabarti, R., Evans, J.F. & Fearnley, G.R.: Effects on platelet stickiness and fibrinolysis of phenformin combined with ethyloestrenol or stanozolol. Lancet 1:591, 1970.
- Chen, R. & Doolittle, R.F.: γ - γ cross-linking sites in human and bovine fibrin. Biochemistry 10:4486, 1971.
- Chirawong, P., Singh Nanra, R. & Kincaid-Smith, P.: Fibrin degradation products and the role of coagulation in "persistent" glomerulonephritis. Ann intern Med 74:853, 1971.
- Christensen, L.R.: Streptococcal fibrinolysis: a proteolytic reaction due to a serum enzyme activated by streptococcal fibrinolysin. J gen Physiol 28:363, 1945.
- Christensen, L.R. & Smith, D.H.: Plasminogen purification by acid extraction. Proc Soc exp Biol Med 74:840, 1950.
- Churg, J. & Grishman, E.: Ultrastructure of immune deposits in renal glomeruli. Ann intern Med 76:479, 1972.
- Claes, G., Svalander, C. & Bergentz, S.-E.: The accumulation and distribution of platelets and fibrin in rejecting dog kidneys. Acta chir scand 139:91, 1973.
- Claeys, H., Molla, A. & Verstraete, M.: Digestion of human plasminogen by human plasmin. IVth Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.

- Clarke, R.L., Orandi, A. & Clifton, E.E.: Induction of fibrinolysis by venous obstruction. *Angiology* 11: 367, 1960.
- Clarkson, A.R., MacDonald, M.K., Petrie, J.J.B., Cash, J.D. & Robson, J.S.: Serum and urinary fibrin/fibrinogen degradation products in glomerulonephritis. *Brit med J* 3:447, 1971.
- Clarkson, A.R., Morton, J.B. & Cash, J.D.: Urinary fibrin/fibrinogen degradation products after renal homotransplantation. *Lancet* 2:1220, 1970.
- Collen, D.: Plasminogen turnover studies in man. IVth Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Collen, D., Tytgat, G., Claeys, H., Verstraete, M. & Wallén, P.: Metabolism of plasminogen in healthy subjects: Effect of tranexamic acid. *J clin Invest* 51:1310, 1972a.
- Collen, D., Tytgat, G.N., Claeys, H. & Piessens, R.: Metabolism and distributions of fibrinogen. I. Fibrinogen turnover in physiological conditions in humans. *Brit J Haemat* 22:681, 1972b.
- Correll, J.T. & Sjoerdsma, A.: Inhibition of a human fibrinolytic system by normal and pathologic sera. *Proc Soc exp Biol Med (N.Y.)* 111:274, 1962.
- Cronberg, S.: Investigations in haemorrhagic disorders with prolonged bleeding time but normal number of platelets. With special reference to platelet adhesiveness. *Acta med scand Suppl* 486, 1968.
- Cucuianu, M., Popescu, T.A. & Hărăgus, S.: Clinical studies concerning inhibition of fibrinolysis in a urokinase activated system; with special reference to atherosclerosis and the proteosynthetic function of the liver. *Rev roum Méd Int* 9, 4:377, 1972.
- Cultrera, G., Cascone, A., Giarola, P. & Gibelli, A.: Effect of 4-aminomethylcyclohexane-carboxylic acid on fibrinogen turnover. *Il Farmaco* 27:174, 1972.
- Davidson, J.F., Conkie, J.A. & McDonald, G.A.: Fibrinolytic enhancement with stanozolol in men with ischaemic heart disease. III Intern. Congr. Soc. Thromb. Haemostasis, Aug 22-26, 1972, Washington, D.C.
- Davis, B.J.: Disc electrophoresis. II. Method and application to human serum proteins. *Ann N Y Acad Sci* 121:404, 1964.
- Davison, A.M., Thomson, D., Macdonald, M.K., Rae, J.K., Uttley, W.S. & Clarkson, A.R.: Identification of intrarenal fibrin deposition. *J clin Path* 26:102, 1973.
- Dechavanne, M., Viala, J.-J., Arnaud, Ph., Creyssel, R. & Lagarde, M.: Activité fibrinolytique des plaquettes. *Nouv Presse Med* 2:653, 1973.

- Denk, H., Schnack, H. & Deutsch, E.: Histochemischer Nachweis fibrinolytischer Aktivität in Leberbiopsien. *Acta Hepato-Splenologica* 17:221, 1970.
- Derechin, M., Johnson, P. & Szuchet, S.: Further studies with human plasminogen. *Biochem J* 84:336, 1962.
- Deutsch, D.G. & Mertz, E.T.: Plasminogen: purification from human plasma by affinity chromatography. *Science* 170:1095, 1970.
- Doleschel, W. & Auerswald, W.: Determination of the molecular weights of uroprotein fractions with urokinase activity by means of molecular sieving. *Med Pharmacol exp* 16:225, 1967.
- Donaldson, G.W.K., Davies, S.H., Darg, A. & Richmond, J.: Coagulation factors in chronic liver disease. *J clin Path* 22:199, 1969.
- Donati, M.B., Vermylen, J. & Verstraete, M.: The staphylococcal clumping test for detection of fibrinogen-like material. *Scand J Haemat Suppl* 13:137, 1971.
- Downie, G.R. & Cliffton, E.E.: Variations in proteolytic activity in serum of animals including man. *Proc Soc exp Biol Med* 73:559, 1950.
- Duckert, F., Jung, E. & Schmerling, D.: A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. *Thromb Diath haemorrh* 5:179, 1960.
- Dudok de Wit, Chr.: Investigations on the inhibitors of the fibrinolytic system. *Thromb Diath haemorrh* 12:105, 1964.
- Dunn, M.S., Murphy, E.A., Salisbury, P.F.: Free and apparent amino acids in deproteinized plasma of normal and uremic (nephrectomized) dogs. *Proc Soc exp Biol Med (N Y)* 92:682, 1956.
- Edgington, T.S. & Plow, E.: Immunochemical approaches to the composition and structural significance of the D:E core of fibrinogen. III Intern. Congr. Thromb. Haemostasis, Washington D.C., Aug 22-26, 1972.
- Edward, N., Young, D.P.-G. & MacLeod, M.: Fibrinolytic activity in plasma and urine in chronic renal disease. *J clin Path* 17:365, 1964.
- Ekelund, H., Hedner, U. & Nilsson, I.M.: Fibrinolysis in newborns. *Acta paediat scand* 59:33, 1970.

- Ekert, H., Barratt, T.M., Chantler, C. & Turner, M.W.: Immunologically reactive equivalents of fibrinogen in sera and urine of children with renal disease. *Arch Dis Childh* 47:90, 1972a.
- Ekert, H., Powell, H. & Muntz, R.: Hypercoagulability in acute glomerulonephritis. *Lancet* 1:965, 1972b.
- Ekert, H., Friedlander, I. & Hardisty, R.M.: The role of platelets in fibrinolysis. Studies on the plasminogen activator and anti-plasmin activity of platelets. *Brit J Haemat* 18:575, 1970.
- Epstein, M.D., Beller, F.K. & Douglas, G.W.: Kidney tissue activator of fibrinolysis in relation to pregnancy. *Obstet Gynec* 32:494, 1968.
- Eriksson, S.: Studies in α_1 -antitrypsin deficiency. *Acta med scand Suppl* 432, 1965.
- Faarvang, H.J. & Lauritsen, O.S.: Increase of trypsin inhibitor in serum during pregnancy. *Nature* 199:290, 1963.
- Fantl, P.: Plasminogen activity of plasma and serum. *Science* 135:787, 1962.
- Fearnley, G.R.: Fibrinolysis. Arnold, London 1965.
- Fearnley, G.R. & Lackner, R.: The fibrinolytic activity of normal blood. *Brit J Haemat* 1:189, 1955.
- Finlayson, J.S., Mosesson, M.W., Bronzert, T.J. & Pisano, J.J.: Human fibrinogen heterogeneities. II. Cross-linking capacity of high solubility catabolic intermediates. *J biol Chem* 247:5220, 1972.
- Flute, P.T.: Fibrinolytic factors as demonstrated by electrophoresis of human blood. *Proc 7th Europ Congr. Soc. Haemat.*, London 1959; part II pp 894-898, 1960.
- Forman, W.B. & Barnhart, M.I.: Cellular site for fibrinogen synthesis. *J amer med Ass* 187:128, 1964.
- Franklin, W.A., Simon, N.M., Potter, E.W. & Krumlovsky, F.A.: The hemolytic-uremic syndrome. *Arch Path* 94:230, 1972.
- Frénoy, J.-P., Razafimahaleo, E. & Bourrillon, R.: Études sur la structure de l' α_2 -macroglobuline humaine. III. Isolement et caractérisation d'une sous-unité. *Biochim Biophys Acta* 257:111, 1972.

- Frimpter, G.W., Thompson, D.D. & Luckey, E.H.: Conjugated amino acids in plasma of patients with uremia. *J clin Invest* 40:1208, 1961.
- Fritz, H., Trautschold, I., Haendle, H. & Werle, E.: Chemistry and biochemistry of proteinase inhibitors from mammalian tissues. *Ann N Y Acad Sci* 146:400, 1968.
- Fuller, T., Olsen, N., Block, A. & Cade, J.: Wegener's granulomatosis. Treatment with heparin in addition to Azathioprine and corticosteroids. *Nephron* 9:225, 1972.
- Furlan, M. & Beck, E.A.: Plasmic degradation of human fibrinogen. I. Structural characterization of degradation products. *Biochim Biophys Acta* 263:631, 1972.
- Furlan, M. & Beck, E.A.: Plasmic degradation of human fibrinogen. II. Further characterization of fragment D. *Biochim Biophys Acta* 310:205, 1973.
- Gaffney, P.J.: Localisation of carbohydrate in the subunits of human fibrinogen and its plasmin induced fragments. *Biochim Biophys Acta* 263:453, 1972a.
- Gaffney, P.J.: F.D.P. *Lancet* 2:1422, 1972b.
- Gaffney, P.J.: The problem of a reference material for fibrinogen/fibrin fragment D produced in vivo. III Intern. Congr. Soc. Thromb. Haemostasis, Washington D.C., Aug 22-26, 1972c.
- Gaffney, P.J. & Brasher, M.: Subunit structure of the plasmin-induced degradation products of cross-linked fibrin. *Biochim Biophys Acta* 295:308, 1973.
- Gaffney, P.J. & Dobos, P.: A structural aspect of human fibrinogen suggested by its plasmin degradation. *FEBS letters* 15:13, 1971.
- Ganguly, P.: A low molecular weight antiplasmin of human blood platelets. *Clin Chim Acta* 39:466, 1972.
- Ganrot, P.O.: Determination of α_2 -macroglobulin as trypsin-protein esterase. *Clin Chim Acta* 14:493, 1966.
- Ganrot, P.O.: Inhibition of plasmin activity by α_2 -macroglobulin. *Clin Chim Acta* 16:328, 1967a.
- Ganrot, P.O.: Studies on serum protease inhibitors with special reference to α_2 -macroglobulin. *Acta Univ Lund* II:2, 1967b.
- Ganrot, P.O.: Crossed immunoelectrophoresis. *Scand J clin Lab Invest* 29, Suppl 124:39, 1972.
- Ganrot, P.O. & Bjerre, B.: α_1 -antitrypsin and α_2 -macroglobulin concentration in serum during pregnancy. *Acta obstet gynec scand* 46:126, 1967.
- Ganrot, P.O. & Niléhn, J.-E.: Competition between plasmin and thrombin for α_2 -macroglobulin. *Clin Chim Acta* 17:511, 1967.

- Ganrot, P.O. & Niléhn, J.-E.: Immunochemical determination of human plasminogen. *Clin Chim Acta* 22:335, 1968.
- Ganrot, P.O. & Scherstén, B.: Serum α_2 -macroglobulin concentration and its variation with age and sex. *Clin Chim Acta* 15:113, 1967.
- Gans, H. & Tan, B.H.: α_1 -antitrypsin, an inhibitor for thrombin and plasmin. *Clin Chim Acta* 17:111, 1967.
- Geiger, W.B.: Involvement of a complementlike factor in the activation of blood protease. *J Immunol* 69:597, 1952.
- Gerbeck, C.M., Yoshikawa, T. & Montgomery, R.: Bovine fibrinogen-heterogeneity of the γ -chains. *Arch Biochem Biophys* 134:67, 1969.
- Gilchrist, G.S. & Liebermann, E.: Haemolytic-uraemic syndrome and heparin therapy. *Lancet* 2:1069, 1969.
- Gilchrist, G.S., Liebermann, E., Ekert, H., Fine, R.N. & Grushkin, C.: Heparin therapy in the haemolytic uraemic syndrome. *Lancet* 1:1123, 1969.
- Goldstein, I.M., Wünschmann, B., Astrup, T. & Henderson, E.S.: Effects of bacterial endotoxin on the fibrinolytic activity of normal human leukocytes. *Blood* 37:447, 1971.
- Gormsen, J.: Fibrinolytic activity. Inhibition of plasminogen activation. *Acta med scand* 172:657, 1962.
- Gottlob, R., Donas, P. & El Nashef, B.: The content of plasminogen, of plasma plasmin inhibitors and the fibrin patterns in arterial and venous thrombi of various age, immunofluorescent investigations. IVth Intern. Congr. Thromb. Haemostasis, Vienna June 19-22, 1973.
- Grob, P.J., Thöni, R. & Schettlin, W.: Fibrinogenabbau-produkte im Urin nach Nierentransplantation. *Schweiz med Wschr* 101:873, 1971.
- Groskopf, W.R., Hsieh, B., Summaria, L. & Robbins, K.C.: Studies on the active center of human plasmin. The serine and histidine residue. *J Biol Chem* 244:359, 1969.
- Gårdlund, B., Kowalska-Loth, B., Gröndahl, N. & Blombäck, B.: Hydrophobic disulfide knots in human fibrinogen. III Intern. Congr. Soc. Thromb. Haemostasis, Washington D.C., Aug 22-26, 1972.
- Haanen, C., Holdrinet, A. & Wijdeveld, P.: Intravascular clotting and acute renal failure. *Scand J Haemat Suppl* 13:337, 1971a.
- Haanen, C., Nováková, I., Wijdeveld, P. & van Liebergen, F.: Significance of fibrin split products in patients with renal failure. *Scand J Haemat Suppl* 13:345, 1971b.

- Habib, R., Courtecuisse, V., Lerclec, F., Mathieu, H. & Royer, P.: Etude anatomo-pathologique de 35 observations de syndrome hemolytique et uremique d l'enfant. Arch franç Pediat 26:391, 1969.
- Hallén, A. & Nilsson, I.M.: Coagulation studies in liver disease. Thromb Diath haemorrh (Stuttg) 11:51, 1964.
- Hamberg, U.: Studies on plasminogen activators. I. Sucrose density gradient distribution of activators from normal and lytic human plasma. Acta chem scand 20:2529, 1966.
- Hamberg, U.: Regulation of kininogenase action by human α_2 -macroglobulin. IVth Intern. Congr. Soc. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Hampton, J.W., Oldham, F.B., Bannerjee, D., Kalmaz, E., Delaney, R. & MacLeod, C.M.: Plasma activator of plasminogen: isolation and characterization in a familial bleeding diathesis. III Intern. Congr. Soc. Thromb. Haemostasis, Aug 22-26, 1972, Washington D.C.
- Hardaway, R.M.: Syndromes of disseminated intravascular coagulation. Thomas ed., Springfield 1966.
- Hatton, M.W.C. & Regoeczi, E.: Studies of the isolation by affinity chromatography and some properties of rabbit and human plasminogens. IVth Intern. Congr. Soc. Thromb. Haemostasis, Vienna June 19-22, 1973.
- Hawiger, J., Niewiarowski, S., Gurewich, V. & Thomas, D.P.: Measurement of fibrinogen and fibrin degradation products in serum by staphylococcal clumping test. J Lab clin Med 75:93, 1970.
- Hawkey, Ch.M. & Stafford, J.L.: A standard clot method for the assay of plasminogen activators, anti-activators, and plasmin. J clin Path 17:175, 1964.
- Hayne, O.A. & Sherman, L.A.: In vivo behavior of fibrinogen fragment D in experimental renal, hepatic and reticuloendothelial dysfunction. Amer J Path 71:219, 1973.
- Hedner, U. & Nilsson, I.M.: Determination of plasminogen in human plasma by a casein method. Thromb Diath haemorrh 14:545, 1965.
- Hedner, U. & Nilsson, I.M.: Clinical experience with determination of fibrinogen degradation products. Acta med scand 189:471, 1971.
- Hedner, U. & Nilsson, I.M.: Parallel determinations of FDP and fibrin monomers with various methods. Thromb Diath haemorrh 28:268, 1972.
- Hedner, U. & Nilsson, I.M.: Maladies du rein et produits de dégradation de la fibrine (PDF). Actualités nephrol 17:257, 1973.
- Heide, K., Heimbürger, N. & Haupt, H.: An inter-alpha trypsin inhibitor of human serum. Clin Chim Acta 11:82, 1965.

- Heimbürger, N.: On the proteinase inhibitors of human plasma with especial reference to antithrombin. Behringwerke AG, Marburg/Lahn, Germany, pp 353-361, 1967.
- Heimbürger, N.: Basic mechanism of action of streptokinase and urokinase. *Thromb Diath haemorrh Suppl* 47:21, 1971.
- Heimbürger, N. & Haupt, H.: Charakterisierung von α_1 X-Glykoprotein als Chymotrypsin-Inhibitor des Humanplasmas. *Clin Chim Acta* 12:116, 1965.
- Hellem, A.J.: The adhesiveness of human blood platelets in vitro. *Scand J clin Lab Invest* 12, Suppl 51, 1960.
- Henriksson, P., Hedner, U., Nilsson, I.M., Boehm, J., Lorand, L. & Robertson, B.: Factor XIII (fibrin stabilizing factor = FSF) in the foetus and the newborn infant. In press 1973.
- Henschen, A. & Edman, P.: Large scale preparation of S-carboxy-methylated chains of human fibrin and fibrinogen and the occurrence of γ -chain variants. *Biochim Biophys Acta* 263:351, 1972.
- Herdman, R.C., Edson, J.R., Pickering, R.J., Fish, A.J., Marker, S. & Good, R.A.: Anticoagulants in renal disease in children. *Amer J Dis Child* 119:27, 1970.
- Herschlein, H.J. & Steichele, D.F.: Immunochemischer Nachweis von Fibrinogenderivaten im Urin bei Ver-
brauchs-koagulopathien. *Thromb Diath haemorrh* 19:248, 1968.
- Hilden, M. & Lauritsen, O.: The fibrinolytic system of plasma after electroshock. *Scand J clin Lab Invest* 28:435, 1971.
- Hultin, E. & Lundblad, G.: Investigations on plasmin. III. On the formation of plasmin from plasminogen. *Acta chem scand* 3:620, 1949.
- Humair, L., Potter, E.V. & Kwaan, H.C.: The role of fibrinogen in renal disease. I. Production of experimental lesions in mice. *J Lab clin Med* 74:60, 1969.
- Hummel, B.W.C., Buck, F.F. & de Renzo, E.C.: Interaction of streptokinase and human plasminogen. *J biol Chem* 241:3474, 1966.
- Hutton, R.A. & O'Shea, M.J.: Haemostatic mechanism in uraemia. *J clin Path* 21:406, 1968.
- Innes, D. & Sevitt, S.: Coagulation and fibrinolysis in injured patients. *J clin Path* 17:1, 1964.
- Ikkala, E. & Nevanlinna, H.R.: Congenital deficiency of fibrin-stabilizing factor. *Thromb Diath haemorrh* 7:567, 1962.
- Isacson, S. & Nilsson, I.M.: Effect of treatment with combined phenformin and ethyloestrenol on the coagulation and fibrinolytic system. *Scand J Haemat* 7:404, 1970.

- Isacson, S. & Nilsson, I.M.: The kidneys and the fibrinolytic activity in the blood. *Thromb Diath haemorrh* 22:211, 1969.
- Isacson, S. & Nilsson, I.M.: Defective fibrinolysis in blood and vein walls in recurrent "idiopathic" venous thrombosis. *Acta chir scand* 138:313, 1972.
- Ito, T., Niwa, T. & Matsui, E.: Fibrinolytic activity in renal disease. *Clin Chim Acta* 36:145, 1972.
- Jacobsen, C.D.: The antiplasmin activity of human plasma and serum and its dependence on ions. *Scand J clin Lab Invest* 16:383, 1964.
- Jacobsen, C.D.: Proteolytic capacity in human plasma. Part II. Genetics, and clinical study. *Scand J clin Lab Invest* 21:227, 1968.
- Jacobsson, K.: Studies on the trypsin and plasmin inhibitors in human blood serum. *Scand J clin Lab Invest Suppl* 14:55, 1955.
- James, K., Johnson, G. & Fudenberg, H.H.: The quantitative estimation of α_2 -macroglobulin in normal pathological and cord sera. *Clin Chim Acta* 14:207, 1966.
- Jamieson, G.A. & Gaffney, Jr, P.J.: Nature of the high molecular weight fraction of fibrinolytic digests of human fibrinogen. *Biochim Biophys Acta* 154:96, 1968.
- Johansson, B.G. & Malmquist, J.: Quantitative immunochemical determination of lysozyme (muramidase) in serum and urine. *Scand J clin Lab Invest* 27:255, 1971.
- Johnson, A.J., Kline, D.L. & Alkjaersig, N.: Assay methods and standard preparations for plasmin, plasminogen and urokinase in purified systems, 1967-1968. *Thromb Diath haemorrh (Stuttg)* 21:259, 1969a.
- Johnson, A.J., Skoza, L. & Tse, A.O.: Studies on plasmin inhibition and plasminogen activation and its inhibition. *Thromb Diath haemorrh (Stuttg) Suppl* 32:105, 1969b.
- Johnson, A.J., Tse, A. & Skoza, L.: Variation in assays of standard SK and plasmin preparations: evidence for and effect of variability of plasmins, plasminogen, and fibrinogen. *Fed Proc* 25:34, 1966.
- Johnson, S.A. & Schneider, C.L.: The existence of anti-fibrinolysin activity in platelets. *Science* 117:229, 1953.
- Kaplan, M.H.: Nature and role of lytic factor in hemolytic streptococcal fibrinolysis. *Proc Soc exp Biol Med* 57:40, 1944.
- Katz, D.H., Unanue, E.R. & Dixon, F.J.: Induction of intravascular coagulation and focal tissue necrosis in rats by administration of anti-connective tissue antibodies. *Amer J Path* 53:835, 1968.

- Katz, J., Lurie, A. & Kaplan, B.: Haemolytic-uraemic syndrome and heparin therapy. *Lancet* 2:700, 1969.
- Katz, J., Lurie, A., Kaplan, B.S., Krawitz, S. & Metz, J.: Coagulation findings in the haemolytic-uraemic syndrome of infancy: similarity to hyperacute renal allograft rejection. *J Pediatr* 78:426, 1971.
- Kawano, T., Morimoto, K. & Uemura, Y.: Urokinase inhibitor in human placenta. *Nature* 217:253, 1968.
- Kawano, T., Morimoto, K. & Uemura, Y.: Partial purification and properties of urokinase inhibitor from human placenta. *J Biochem* 67:333, 1970.
- Kawano, T. & Uemura, Y.: Inhibition of tissue activator by urokinase inhibitor. *Thromb Diath haemorrh* 26: 129, 1971.
- Kelley, V.C.: Acute phase reactants; serum nonglycosamine polysaccharides in patients with rheumatic fever and related conditions. *J Pediatr* 40:405, 1952.
- Kierulf, P. & Abildgaard, U.: Studies on soluble fibrin in plasma. I. N-terminal analysis of a modified fraction I (Cohn) from normal and thrombin-incubated plasma. *Scand J clin Lab Invest* 28:231, 1971.
- Kincaid-Smith, P.: Anticoagulants in renal disease. *Amer Heart J* 77:840, 1969.
- Kincaid-Smith, P., Laver, M.C., Fairley, K.F. & Mathews, D.C.: Dipyridamole and anticoagulants in renal disease due to glomerular and vascular lesions. A new approach to therapy. *Med J Austr* 1:145, 1970.
- Kincaid-Smith, P., Saker, B.M. & Fairley, K.F.: Anticoagulants in "irreversible" acute renal failure. *Lancet* 2:1360, 1968.
- Kleinknecht, D. & Kanfer, A.: La coagulation intravasculaire. Son rôle en pathologie rénale. *Nouv Presse Méd* 2:925, 1973.
- Kline, D.L.: The purification and crystallization of plasminogen (profibrinolysin). *J biol Chem* 204:949, 1953.
- Kline, D.L. & Fishman, J.B.: Proactivator function of human plasmin as shown by lysine esterase assay. *J biol Chem* 236:2807, 1961a.
- Kline, D.L. & Fishman, J.B.: Improved procedure for the isolation of human plasminogen. *J biol Chem* 236: 3232, 1961b.
- Kline, D.L. & Ts'ao, C.-H.: Activation of human plasminogen by streptokinase in the absence of plasmin-SK activator. *Amer J Physiol* 220:440, 1971.
- Koffler, D. & Paronetto, F.: Immunofluorescent localization of immunoglobulins, complement, and fibrinogen in human diseases. II. Acute, subacute and chronic glomerulonephritis. *J clin Invest* 44:1665, 1965.

- Koffler, D. & Paronetto, F.: Fibrinogen deposition in acute renal failure. *Amer J Path* 49:383, 1966.
- Kok, P. & Astrup, T.: Isolation and purification of a tissue plasminogen activator and its comparison with urokinase. *Biochemistry* 8:79, 1969.
- Konishi, K. & Takagi, T.: Activation of the fibrin stabilizing factor by trypsin and some of its properties after activation. *J Biochem* 65:281, 1969.
- Kopec, M., Budzynski, A., Stachurska, J., Wegrzynowicz, Z. & Kowalski, E.: Studies on the mechanism of interference by fibrinogen degradation products (FDP) with the platelet function. Role of fibrinogen in the platelet atmosphere. *Thromb Diath haemorrh* 15:476, 1966.
- Kowalski, E.: Fibrinogen derivatives and their biologic activities. *Sem Hemat* 5:45, 1968.
- Kröll, J.: On the immunoelectrophoretical identification and quantitation of serum proteins. *Scand J clin Lab Invest* 22:79, 1968.
- Kucinski, C.S., Fletcher, A.P. & Sherry, S.: Effect of urokinase antiserum on plasminogen activators: Demonstration of immunologic dissimilarity between plasma plasminogen activator and urokinase. *J clin Invest* 47:1238, 1968.
- Kunitz, M. & Northrop, J.H.: Isolation from beef pancreas of crystalline trypsinogen, trypsin, a trypsin inhibitor, and in inhibitor-trypsin compound. *J gen Physiol* 19:991, 1936.
- Kwaan, H.C. & Astrup, T.: Demonstration of cellular fibrinolytic activity by the histochemical fibrin slide technique. *Lab Invest* 17:140, 1967.
- Kwaan, H.C. & Fischer, S.: Localization of fibrinolytic activity in kidney tissues. *Fed Proc* 24:387, 1965.
- Kwaan, H.C., Lo, R. & McFadzean, A.J.S.: Antifibrinolytic activity in primary carcinoma of the liver. *Clin Sci* 18:251, 1959.
- Kwaan, H.C. & Suwanwela, N.: Inhibitors of fibrinolysis in platelets in polycythaemia vera and thrombocytosis. *Brit J Haemat* 21:313, 1971.
- Künzer, W. & Aalam, F.: Zur Heparinbehandlung des akuten hämolytisch-urämischen Syndroms. *Klin Wschr* 42:820, 1964.
- Künzer, W. & Lütgemeier, J.: Zur entwicklungsbedingten Abhängigkeit des Plasmagehaltes an Fibrin-stabilisierendem Faktor (FSF). *Ann Pediat* 204:232, 1965.
- Ladehoff, A.A.: The content of plasminogen activator in the human urinary tract. *Scand J clin Lab Invest* 12:136, 1960.
- Laki, K. & Lorand, L.: On the solubility of fibrin clots. *Science* 108:280, 1948.

- Larrieu, M.J.: Action of fibrinogen degradation products and fibrin monomer soluble complexes on platelet aggregation. *Scand J Haemat Suppl* 13:273, 1971.
- Larrieu, M.J., Rigollot, C. & Marder, V.J.: Comparative effects of fibrinogen degradation fragments D and E on coagulation. *Brit J Haemat* 22:719, 1972.
- Larsson, S.O., Hedner, U. & Nilsson, I.M.: On coagulation and fibrinolysis in conservatively treated chronic uraemia. *Acta med scand* 189:433, 1971a.
- Larsson, S.O., Hedner, U. & Nilsson, I.M.: On coagulation and fibrinolysis in acute renal insufficiency. *Acta med scand* 189:443, 1971b.
- Larsson, S.O., Hedner, U. & Nilsson, I.M.: On fibrinolytic split products in serum and urine in uraemia. *Scand J Urol Nephrol* 5:234, 1971c.
- Larsson, S.O., Lindergård, B., Henriksson, H., Hedner, U. & Nilsson, I.M.: A case of acute glomerulonephritis and severe uraemia treated with heparin and corticosteroids. *Scand J Urol Nephrol* 5:291, 1971d.
- Lassen, M.: Heat denaturation of plasminogen in the fibrin plate method. *Acta physiol scand* 27:371, 1952.
- Latallo, Z.S.: Products of fibrin/-ogen/proteolysis. III Intern. Congr. Thromb. Haemostasis, Washington, D.C., Aug 22-26, 1972.
- Latallo, Z.S., Fletcher, A.P., Alkjaersig, N. & Sherry, S.: Inhibition of fibrin polymerization by fibrinogen proteolysis products. *Amer J Physiol* 202:681, 1962.
- Laurell, C.B.: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Analyt Biochem* 15:45, 1966.
- Laurell, C.-B. & Eriksson, S.: The electrophoretic α_1 -globulin pattern of serum in α_1 -antitrypsin deficiency. *Scand J clin Lab Invest* 15:132, 1963.
- Laurell, C.-B. & Eriksson, S.: The serum α_1 -antitrypsin in families with hypo- α_1 -antitrypsinemia. *Clin Chim Acta* 11:395, 1965.
- Lauritsen, O.S.: Urokinase inhibitor in human plasma. *Scand J clin Lab Invest* 22:314, 1968.
- Lauritsen, O.S.: Studies on proactivator activity in human blood. *Scand J clin Lab Invest* 23:129, 1969a.
- Lauritsen, O.S.: The fibrinolytic enzyme system of human blood plasma in pregnancy. *Scand J clin Lab Invest* 23:191, 1969b.
- Lauritsen, O.: The fibrinolytic enzyme system and trypsin inhibitor as measured by the casein method. *Danish med Bull* 17, Suppl II, 1970.
- Lesuk, A., Terminiello, L. & Traver, J.H.: Crystalline human urokinase: some properties. *Science* 147:880, 1965.

- Lewis, J.H. & Ferguson, J.H.: Studies on a proteolytic enzyme system of blood. IV. Activation of profibrinolysin by serum fibrinolysokinase. *Proc Soc exp Biol Med* 78:184, 1951.
- Lewis, J.H., Szeto, I.L.F., Ellis, L.D. & Bayer, W.L.: An acquired inhibitor to coagulation factor XIII. *Johns Hopk Med J* 120:401, 1967.
- Lieberman, E.: Hemolytic-uremic syndrome. *J Ped* 80:1, 1972.
- Ling, C.-M., Summaria, L. & Robbins, K.: Mechanism of formation of bovine plasminogen activator from human plasmin. *J Biol Chem* 240:4213, 1965.
- Ling, C.-M., Summaria, L. & Robbins, K.C.: Isolation and characterization of bovine plasminogen activator from a human plasminogen-streptokinase mixture. *J Biol Chem* 242:1419, 1967.
- Ljungqvist, U., Bergentz, S.-E., Leandoer, L. & Nilsson, I.M.: Coagulation and fibrinolysis after renal transplantation. *Scand J Urol Nephrol* 3:23, 1969.
- Lorand, L.: A study of the solubility of fibrin clots in urea. *Hung Acta Physiol* 1:192, 1948.
- Lorand, L.: Fibrin clots. *Nature* 166:694, 1950.
- Lorand, L.: 'Fibrino-peptide': New aspects of the fibrinogen-fibrin transformation. *Nature* 167:992, 1951.
- Lorand, L.: Physiological crosslinking of fibrin. *Thromb Diath haemorrh Suppl* 39:75, 1970.
- Lorand, L., Downey, J., Gotoh, T., Jacobsen, A. & Tokura, S.: The transpeptidase system which crosslinks fibrin by γ -glutamyl- ϵ -lysine bonds. *Biochem Biophys Res Comm* 31:222, 1968.
- Lorand, L. & Konishi, K.: Activation of the fibrin stabilizing factor of plasma by thrombin. *Arch Biochem Biophys* 105:58, 1964.
- Lorand, L., Maldonado, N., Fraclera, J., Atencio, A.C., Robertson, B. & Urayama, T.: Haemorrhagic syndrome of autoimmune origin with a specific inhibitor against fibrin stabilizing factor (factor XIII). *Brit J Haemat* 23:17, 1972.
- Lorand, L., Urayama, T., Atencio, A.C. & Hsia, D.Y.-Y.: Inheritance of deficiency of fibrin-stabilizing factor (factor XIII). *Amer J hum Genet* 22:89, 1970.
- Losowski, M.S. & Walls, W.D.: Mechanisms of acquired defects of the stabilization of fibrin. *Brit J Haemat* 21:377, 1971.
- Luke, R.G., Siegel, R.R., Talbert, W. & Holland, N.: Heparin treatment for post-partum renal failure with microangiopathic haemolytic anaemia. *Lancet* 2:750, 1970.
- Lüscher, E.F.: Ein fibrinstabilisierender Faktor aus Thrombocyten. *Schweiz med Wschr* 87:1220, 1957.

- McClintock, D.K. & Bell, P.H.: The mechanism of activation of human plasminogen by streptokinase. *Biochem Biophys Res Comm* 43:694, 1971.
- McCluskey, R.T. & Klassen, J.: Immunologically mediated glomerular, tubular and interstitial renal disease. *New Engl J Med* 288:564, 1973.
- McConnel, D., Johnson, J.G., Joung, I. & Holemans, R.: Localization of plasminogen activator activity in kidney tissue. *Lab Invest* 15:980, 1966.
- McDonagh, J. & McDonagh, Jr, R.P.: Factor XIII from human platelets: effect on fibrin crosslinking. *Thrombosis Research* 1:147, 1972.
- McDonagh, J., McDonagh, R.P., Delâge, J.-M. & Wagner, R.H.: Factor XIII in human plasma and platelets. *J clin Invest* 48:940, 1969a.
- McDonagh, J., Kiesselbach, T.H. & Wagner, R.H.: Factor XIII and antiplasmin activity in human platelets. *Amer J Physiol* 216:508, 1969b.
- McDonagh, J., Messel, H., McDonagh, Jr, R.P., Murano, G. & Blombäck, B.: Molecular weight analysis of fibrinogen and fibrin chains by an improved sodium dodecyl sulfate gel electrophoresis method. *Biochim Biophys Acta* 257:135, 1972.
- McDonagh, J. & Wagner, R.H.: Separation of plasma and platelet factor XIII by gel filtration. *Amer J Physiol* 219:1555, 1970.
- Macfarlane, R.G. & Biggs, R.: Fibrinolysis. Its mechanism and significance. *Blood* 3:1167, 1948.
- McGale, E.H., Pickford, J.C. & Aber, G.M.: Quantitative changes in plasma amino acids in patients with renal disease. *Clin Chim Acta* 38:395, 1972.
- McKay, D.G.: Disseminated intravascular coagulation. An intermediary mechanism of disease. Hoeber Medical Division, Harper & Row, Publishers New York, Everton, London, 1965.
- McKay, D.G. & Margaretten, W.: Disseminated intravascular coagulation in virus diseases. *Arch intern Med* 120:129, 1967.
- McKee, P.A., Mattock, P. & Hill, R.L.: Subunit structure of human fibrinogen, soluble fibrin, and cross-linked insoluble fibrin. *Proc nat Acad Sci* 66:738, 1970.
- MacLeod, M., Stalker, A.L. & Ogston, D.: Fibrinolytic activity of lung tissue in renal failure. *Lancet* 1:191, 1962.
- McNicol, G.P., Barakat, A.A. & Douglas, A.S.: Plasma fibrinolytic activity in renal disease. *Scot med J* 10:189, 1965.

- McNicol, G.P., Gale, S.B. & Douglas, A.S.: In-vitro and in-vivo studies of a preparation of urokinase. *Brit med J* 1:909, 1963.
- McNicol, G.P., Prentice, C.R.M., Briggs, J.D. & Pidgeon, C.: Fibrinogen degradation products (F.D.P.) in renal disease: Estimation and significance of F.D.P. in urine. *Scand J Haemat Suppl* 13:329, 1971.
- Malofiejew, M.: The biological and pharmacological properties of some fibrinogen degradation products. *Scand J Haemat Suppl* 13:303, 1971.
- Mann, R.D.: Method of estimating the inhibitory effect of plasma or serum upon urokinase-activated fibrinolysis. *J clin Path* 19:233, 1966.
- Mann, R.D.: Effect of age, sex and diurnal variations on the human fibrinolytic system. *J clin Path* 20:223, 1967.
- Marder, V.J.: Fibrinogen and fibrin degradation products. Physicochemical and physiological considerations. *Thromb Diath haemorrh Suppl* 47:85, 1971.
- Marder, V.J., Budzynski, A.Z. & James, H.L.: High molecular weight derivatives of human fibrinogen produced by plasmin. III. Their NH₂-terminal amino acids and comparison with the NH₂-terminal disulphide knot. *J biol Chem* 247:4775, 1972.
- Marder, V.J. & Shulman, N.R.: High molecular weight derivatives of human fibrinogen produced by plasmin. II. Mechanism of their anticoagulant activity. *J biol Chem* 244:2120, 1969.
- Marder, V.J., Shulman, N.R. & Carroll, W.R.: The importance of intermediate degradation products of fibrinogen in fibrinolytic hemorrhage. *Trans Ass amer Physicians Phil* 80:156, 1967.
- Marder, V.J., Shulman, N.R. & Carroll, W.R.: High molecular weight derivatives of human fibrinogen produced by plasmin. I. Physicochemical and immunological characterization. *J biol Chem* 244:2111, 1969.
- Markwardt, F., Klöcking, H.P. & Landmann, H.: Vergleichende Untersuchungen über Antifibrinolytika. *Thromb Diath haemorrh* 15:561, 1967.
- Matacic, S. & Loewy, A.G.: The identification of isopeptide crosslinks in insoluble fibrin. *Biochem Biophys Res Comm* 30:356, 1968.
- Matis, P. & Mörl, F.K.: Trasylol therapy in the surgical patient. *Ann N Y Acad Sci* 146:715, 1968.
- Mauer, S.M., Sutherland, D.E.R., Howard, R.J., Fish, A.J., Najarian, J.S. & Michael, A.F.: The glomerular mesangium. III. Acute immune mesangial injury: a new model of glomerulonephritis. *J exp Med* 137:553, 1973.

- Mehl, J.W., O'Connell, W. & De Groot, J.: Macroglobulin from human plasma which forms an enzymatically active component with trypsin. *Science* 145:821, 1964.
- Melliger, E.J.: Detection of fibrinogen degradation products by use of antibody coated latex particles. *Thromb Diath haemorrh* 23:211, 1970.
- Merskey, C.: Defibrination. *New Engl J Med* 282:688, 1970.
- Merskey, C.: Discussion. *Scand J Haemat Suppl* 13:373, 1971.
- Merskey, C., Lalezari, P. & Johnson, A.J.: Tanned red cell hemagglutination inhibition immunoassay for fibrinogen-fibrin-related antigen in human serum. *Scand J Haemat Suppl* 13:83, 1971.
- Miesch, F., Bieth, J. & Metais, P.: The α_1 -antitrypsin and α_2 -macroglobulin content and the protease-inhibiting capacity of normal and pathological sera. *Clin Chim Acta* 31:231, 1971.
- Miller, L.L. & John, D.W.: Factors affecting net fibrinogen biosynthesis by the isolated perfused rat liver. *Thromb Diath haemorrh Suppl* 39:127, 1970.
- Mills, D.A.: Molecular model for the proteolysis of human fibrinogen by plasmin. *Biochim Biophys Acta* 263:619, 1972.
- Mills, D. & Karparkin, S.: Heterogeneity of human fibrinogen. *Biochem Biophys Res Comm* 40:206, 1970.
- Mole, R.H.: Fibrinolysin and the fluidity of the blood post mortem. *J path Bact* 60:413, 1948.
- Moll, F.C., Sunden, S.F. & Brown, J.R.: Partial purification of the serum trypsin inhibitor. *J biol Chem* 233:121, 1958.
- Monnens, L. & Schretlen, E.: Haemolytic-uraemic syndrome. *Lancet* 2:735, 1968.
- Morris, R.H., Vassalli, P., Beller, F.K. & McCluskey, R.T.: Immunofluorescent studies of renal biopsies in the diagnosis of toxemia of pregnancy. *Obstet Gynec* 24:32, 1964.
- Mosesson, M.W.: The preparation of human fibrinogen free of plasminogen. *Biochim Biophys Acta* 57:204, 1962.
- Mosesson, M.W., Alkjaersig, N., Sweet, B. & Sherry, S.: Human fibrinogen of relatively high solubility. Comparative biophysical, biochemical, and biological studies with fibrinogen of lower solubility. *Biochemistry* 6:3279, 1967.
- Mosesson, M.W., Finlayson, J.S. & Galanakis, D.K.: Studies of the recovery of fragment D from plasmic digests of fibrinogen. IV Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.

- Mosesson, M.W., Finlayson, J.S., Umfleet, R.A. & Galanakis, D.: Human fibrinogen heterogeneities. I. Structural and related studies of plasma fibrinogens which are high solubility catabolic intermediates. *J Biol Chem* 247:5210, 1972a.
- Mosesson, M.W., Finlayson, J.S. & Umfleet, R.A.: Human fibrinogen heterogeneities. III. Identification of γ chain variants. *J Biol Chem* 247:5223, 1972b.
- Mosesson, M.W. & Sherry, S.: The preparation and properties of human fibrinogen of relatively high solubility. *Biochemistry* 5:2829, 1966.
- Müllertz, S.: Fibrinolytic activity of human blood after death. *Acta physiol scand* 27:265, 1952.
- Müllertz, S.: The mechanism of fibrinolysis in blood. *Commun. IIe Congr. Int. Biochim., Paris, 1953 a*, pp 414-415.
- Müllertz, S.: The action of plasmin on fibrin and fibrinogen in blood. *Acta physiol scand* 28:29, 1953b.
- Müllertz, S.: The plasmin activator system in human blood. *Proc. 1st Intern. Conf. Thrombosis and Embolism, Basel 1954a*, p 79.
- Müllertz, S.: Effect of carboxylic and amino acids on fibrinolysis produced by plasmin, plasminogen activator and proteases. *Proc Soc exp Biol Med* 85:326, 1954b.
- Müllertz, S.: Formation and properties of the activator of plasminogen and of human and bovine plasmin. *Biochem J* 61:424, 1955.
- Müllertz, S.: Mechanism of activation and effect of plasmin in blood. *Acta physiol scand Suppl* 130, 38:33, 1956.
- Müllertz, S.: Activation of plasminogen. *Ann N Y Acad Sci* 68:38, 1957.
- Müllertz, S.: Molecular forms of plasmin and protease inhibitors in human fibrinolytic post-mortem plasma. *Scand J clin Lab Invest* 30:369, 1972.
- Müllertz, S. & Lassen, M.: An activator system in blood indispensable for formation of plasmin by streptokinase. *Proc Soc exp Biol Med* 82:264, 1953.
- Naish, P., Peters, D.K. & Shackman, R.: Increased urinary fibrinogen derivatives after renal allo-transplantation. *Lancet* 1:1280, 1973.
- Naish, P.F., Sevvitt, L.H., Pitney, W.R. & Peters, D.K.: Fibrinogen derivatives in renal disease. *Clin Sci* 39:15, 1970.
- Niewiarowski, S., Prokopowicz, J., Poplawski, A. & Worowski, K.: Inhibition of dog fibrinolytic system in experimental tubular necrosis of kidney. *Experientia* 20:101, 1964.

- Nilēhn, J.-E.: Separation and estimation of "split products" of fibrinogen and fibrin in human serum. *Thromb Diath haemorrh* 18:487, 1967a.
- Nilēhn, J.-E.: Influence of split products of fibrinogen on results of blood coagulation tests and platelet adhesiveness. *Scand J Haemat* 4:430, 1967b.
- Nilēhn, J.-E.: Quantitative immunochemical determination of degradation products of fibrinogen and/or fibrin with high voltage electrophoresis. *Scand J Haemat Suppl* 13:215, 1971.
- Nilēhn, J.-E. & Ganrot, P.O.: Plasmin, plasmin inhibitors and degradation products of fibrinogen in human serum during and after intravenous infusion of streptokinase. *Scand J clin Lab Invest* 20:113, 1967.
- Nilēhn, J.-E. & Nilsson, I.M.: Demonstration of fibrinolytic split products in human serum by an immunological method in spontaneous and induced fibrinolytic states. *Scand J Haemat* 1:313, 1964.
- Nilsson, I.M.: Fibrinolysis and fibrinolytic degradation products in renal disease. *Coagulation* 3:215, 1970.
- Nilsson, I.M.: Blödnings- och trombossjukdomar. *Almqvist & Wiksell* 1971, p 212.
- Nilsson, I.M., Blombäck, M. & von Francken, I.: On an inherited autosomal hemorrhagic diathesis with anti-haemophilic globulin (AHG) deficiency and prolonged bleeding time. *Acta med scand* 159:35, 1957a.
- Nilsson, I.M., Skanse, B. & Gydell, K.: Fibrinolysis in Boeck's sarcoid. *Acta med scand* 159:463, 1957b.
- Nilsson, I.M., Blombäck, M. & Ramgren, O.: Haemophilia in Sweden. I. Coagulation studies. *Acta med scand* 170:665, 1961a.
- Nilsson, I.M., Krook, H., Sternby, N.H., Söderberg, E. & Söderström, N.: Severe thrombotic disease in a young man with bone marrow and skeletal changes and with a high content of an inhibitor in the fibrinolytic system. *Acta med scand* 169:323, 1961b.
- Nilsson, I.M. & Kullander, S.: Coagulation and fibrinolytic studies during pregnancy. *Acta obstet gynec scand* 46:273, 1967.
- Nilsson, I.M. & Olow, B.: Determination of fibrinogen and fibrinogenolytic activity. *Thromb Diath haemorrh (Stuttg)* 8:297, 1962a.
- Nilsson, I.M. & Olow, B.: Fibrinolysis induced by streptokinase in man. *Acta chir scand* 123:247, 1962b.
- Nilsson, I.M. & Pandolfi, M.: Fibrinolytic response of the vascular wall. *Thromb Diath haemorrh Suppl* 40:231, 1970.
- Nilsson, I.M., Pandolfi, M. & Robertson, B.: Properties of fibrinolytic activators appearing after venous stasis and intravenous injection of nicotinic acid. *Coagulation* 3:13, 1970.

- Nilsson, I.M., Sjoerdsma, A. & Waldenström, J.: Antifibrinolytic activity and metabolism of E-aminocaproic acid in man. *Lancet* 1:1322, 1960.
- Noordhoek Hegt, V. & Brakman, P.: Inhibition of fibrinolysis in human tissue as revealed by a modified fibrin slide technique. IVth Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Norman, P.S.: Studies of the plasmin system. II. Inhibition of plasmin by serum or plasma. *J exp Med* 108:53, 1958.
- Norman, P.S. & Hill, B.M.: Studies of the plasmin system. III. Physical properties of the two inhibitors in plasma. *J exp Med* 108:639, 1958.
- Nussbaum, M. & Morse, B.S.: Plasma fibrin stabilizing factor activity in various diseases. *Blood* 23:669, 1964.
- Nussenzweig, V., Seligmann, M., Pelmont, J. & Grabar, P.: Les produits de dégradation du fibrinogène humain par la plasmine. I. Séparation et propriétés physicochimiques. *Ann Inst Pasteur* 100:377, 1961a.
- Nussenzweig, V., Seligmann, M. & Grabar, P.: Les produits de dégradation du fibrinogène humain par la plasmine. II. Étude immunologique: Mise en évidence d'anticorps antifibrinogène natif possédant des spécificités différentes. *Ann Inst Pasteur* 100:490, 1961b.
- Ogston, D., Murray, J., Crawford, G.P.M. & Douglas, A.S.: Studies on a platelet inhibitor of plasminogen activators. IVth Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Ogston, D., Ogston, C.M. & Fullerton, H.W.: The plasminogen content of thrombi. *Thromb Diath haemorrh* 15:220, 1966.
- Okamoto, S., Nagasawa, S., Takagi, E., Tsukada, Y., Koyoi, M. & Sato, M.: Ref. to British Patent Specification 770:693, 1957.
- Okamoto, S. & Okamoto, U.: Amino-methyl-cyclohexanecarboxylic acid: AMCHA. A new potent inhibitor of the fibrinolysis. *Keio J Med* 11:105, 1962.
- Olow, B.: Postoperative changes in the coagulation factors and the fibrinolytic system. *Scand J clin Lab Invest* 16:30, 1964.
- Oncley, J.L., Melin, M., Richert, D.A., Cameron, J.W. & Gross, P.M.: The separation of the antibodies isoagglutinins, prothrombin, plasminogen and β_1 -lipoprotein into subfractions of human plasma. *J amer chem Soc* 71:541, 1959.
- den Ottolander, G.J.H., Leijnse, B. & Cremer-Elfrink, H.M.J.: Plasmatic and thrombocytic antiplasmins and anti-activators. II. *Thromb Diath haemorrh* 21:26, 1969a.

- den Ottolander, G.J.H., Leijnse, B. & Cremer-Elfrink, H.M.J.: Plasmatic and platelet anti-plasmins and anti-activators. III. Results in normal people, in patients and in those with venous thrombosis. *Thromb Diath haemorrh* 21:35, 1969b.
- Owren, P.A.: Prothrombin and accessory factors. *Clinical significance. Amer J Med* 14:201, 1953.
- Owren, P.A. & Aas, K.: The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. *Scand J clin Lab Invest* 3:201, 1951.
- Painter, R.H.: The plasminogen activator from kidney tissue cultures. *Intern. Symp. Anticoagulants and Fibrinolysis*. Ed. McMillan & Mustard, pp 351-359, 1961.
- Painter, R.H. & Charles, A.F.: Characterization of a soluble plasminogen activator from kidney cell cultures. *Amer J Physiol* 202:1125, 1962.
- Pandolfi, M.: Localization of fibrinolytic activity in the developing rat eye. *Arch Ophthal* 78:512, 1967.
- Pandolfi, M.: Studies on fibrinolysis in some tissues and in aqueous humor. Thesis, Berlingska Boktryckeriet, Lund, 1969.
- Pandolfi, M.: Persistence of fibrinolytic activity in fragments of human veins cultured in vitro. *Thromb Diath haemorrh* 24:43, 1970.
- Pandolfi, M.: Histochemistry and assay of plasminogen activator(s). *Europ J clin Biol Res* 17:254, 1972.
- Pandolfi, M., Bergentz, S.-E., Claes, G. & Ljungqvist, U.: Localization of fibrinolytic activity in healthy and diseased renal tissue. 6th Europ. Conf. Micro-circulation, Aalborg 1970, pp 88-93, 1971.
- Pandolfi, M., Isacson, S. & Nilsson, I.M.: Low fibrinolytic activity in the walls of veins in patients with thrombosis. *Acta med scand* 186:1, 1969.
- Paraskevas, M., Nilsson, I.M. & Martinsson, G.: A method for determining serum inhibitors of plasminogen activation. *Scand J clin Lab Invest* 14:138, 1962.
- Paronetto, F.: Immunocytochemical observations on the vascular and renal glomerular lesions of malignant nephrosclerosis. *Amer J Path* 46:901, 1965.
- Paronetto, F. & Koffler, D.: Immunofluorescent localization of immunoglobulins, complement and fibrinogen in human diseases. I. Systemic lupus erythematosus. *J clin Invest* 44:1657, 1965.
- Paronetto, F. & Strauss, L.: Immunocytochemical observations in periarteritis nodosa. *Ann intern Med* 56:289, 1962.
- Pasquini, R. & Hershegold, E.J.: Effects of plasmin on human factor VIII (AHF). *Blood* 41:105, 1973.

- Phillips, L.L., Weiss, R. & Wolf, Ch.: Coagulation and fibrinolytic studies following use of proteinase inhibitors in prostatic surgery. *Ann N Y Acad Sci* 146:737, 1968.
- Pisano, J.J., Finlayson, J.S., Bronzert, T.J. & Peyton, M.P.: ϵ -(γ -glutamyl) lysine crosslink formation in fibrin prepared under various conditions. III Congr. Intern. Soc. Thromb. Haemostasis, Washington D.C., Aug 22-26, 1972.
- Pisano, J.J., Finlayson, J.S. & Peyton, M.P.: Cross-link in fibrin polymerized by factor XIII: ϵ -(γ -glutamyl) lysine. *Science* 160:892, 1968.
- Pizzo, S.V., Schwartz, M.L., Hill, R.L. & McKee, P.A.: The effect of plasmin on the subunit structure of human fibrin. *J biol Chem* 248:4574, 1973a.
- Pizzo, S.V., Taylor, L.M., Schwartz, M.L., Hill, R.L. & McKee, P.A.: Subunit structure of fragment D from fibrinogen and cross-linked fibrin. *J biol Chem* 248:4584, 1973b.
- Pizzo, S.V., Schwartz, M.L., Hill, R.L. & McKee, P.A.: The effect of plasmin on the subunit structure of human fibrinogen. *J biol Chem* 247:636, 1972.
- Pizzo, S.V., Schwartz, M.L., Mattock, P., Hill, R.L. & McKee, P.A.: Correlation of clottability of purified human fibrinogen with its digestion by purified human plasmin. *J Lab clin Med* 76:892, 1970.
- Ploug, J. & Kjeldgaard, N.O.: Urokinase, an activator of plasminogen from human urine. I. Isolation and properties. *Biochem Biophys Acta* 24:278, 1957.
- Plow, E. & Edgington, T.S.: Molecular events responsible for modulation of neoantigenic expression: the cleavage-associated neoantigen of fibrinogen. *Proc nat Acad Sci* 69:208, 1972.
- Plow, E. & Edgington, T.S.: Immunobiology of fibrinogen. Emergence of neoantigenic expressions during physiologic cleavage in vitro and in vivo. *J clin Invest* 52:273, 1973.
- Prentice, C.R.M., Hassanein, A.A., McNicol, G.P. & Douglas, A.S.: Studies on blood coagulation, fibrinolysis and platelet function following exercise in normal and splenectomized people. *Brit J Haemat* 23:541, 1972.
- Preston, F.E., Malia, R.G., Blackburn, E.K. & Platts, M.: FDP in glomerulonephritis. *Brit med J* 4:171, 1971.
- Pugatch, E.M.J., Foster, E.A., Macfarlane, D.E. & Poole, J.C.: The extraction and separation of activators and inhibitors of fibrinolysis from bovine endothelium and mesothelium. *Brit J Haemat* 18:669, 1970.

- Rabiner, S.F., Goldfine, I.D., Hart, A., Summaria, L. & Robbins, K.C.: Radioimmunoassay of human plasminogen and plasmin. *J Lab clin Med* 74:265, 1969.
- Rammer, L.: Intravascular coagulation, fibrinolysis inhibition and posttraumatic renal failure. *Acta Universitatis Upsaliensis* 116, 1972.
- Ratnoff, O.D.: Studies on a proteolytic enzyme in human plasma. II. Some factors influencing the enzymes activated by chloroform and by streptococcal fibrinolysin. *J exp Med* 87:211, 1948.
- Ratnoff, O.D. & Potts, A.M.: The accelerating effect of calcium and other cations on the conversion of fibrinogen to fibrin. *J clin Invest* 33:206, 1954.
- Rayner, H., Paraskevas, F., Israels, L.G. & Israels, E.D.: Fibrinogen breakdown products: Identification and assay in serum and urine. *J Lab clin Med* 74:586, 1969.
- Reddy, K.N.N. & Markus, G.: Mechanism of activation of human plasminogen by streptokinase. Presence of active center in streptokinase-plasminogen complex. *J biol Chem* 247:1683, 1972.
- Remmert, L.F. & Cohen, P.: Partial purification and properties of a proteolytic enzyme of human serum. *J biol Chem* 181:431, 1949.
- de Renzo, E.C., Boggiano, E., Barg, W.F. & Buck, F.F.: Interaction of streptokinase and human plasminogen. IV. Further gel electrophoretic studies on the combination of streptokinase with human plasminogen or human plasmin. *J biol Chem* 242:2428, 1967.
- Rickli, E.E. & Cuendet, P.A.: Isolation of plasmin-free human plasminogen with N-terminal glutamic acid. *Biochim Biophys Acta* 250:447, 1971.
- Rickli, E.E. & Otavsky, W.I.: New aspects of the activation of human plasminogen. IVth Intern. Congr. Thromb. Haemostasis, Vienna June 19-22, 1973.
- Rimon, A., Shamash, Y. & Shapiro, B.: The plasmin inhibitor of human plasma. IV. Its action on plasmin, trypsin, chymotrypsin, and thrombin. *J biol Chem* 241:5102, 1966.
- Rinderknecht, H. & Geokas, M.C.: Role for α_2 -macroglobulin in haemostatic balance. *Nature* 239:116, 1972.
- Robbins, K.C., Bernabe, P., Arzadon, L. & Summaria, L.: The primary structure of human plasminogen. II. The histidine loop of human plasmin: light (B) chain active center histidine sequence. *J biol Chem* 248:1631, 1973.
- Robbins, K.C. & Summaria, L.: Purification of human plasminogen and plasmin by gel filtration on Sephadex and chromatography on diethylaminoethyl-Sephadex. *J biol Chem* 238:952, 1963.

- Robbins, K.C. & Summaria, L.: Biochemistry and fibrinolysis. *Thromb Diath haemorrh Suppl* 47:9, 1971.
- Robbins, K.C. & Summaria, L.: Isoelectric focusing of human plasminogen, plasmin, and derived heavy (A) and light (B) chains. *Ann N Y Acad Sci* 209:397, 1973.
- Robbins, K.C., Summaria, L., Elwyn, D. & Barlow, G.H.: Further studies on the purification and characterization of human plasminogen and plasmin. *J biol Chem* 240:541, 1965.
- Robbins, K.C., Summaria, L., Hsieh, B. & Shah, R.J.: The peptide chains of human plasmin. Mechanism of activation of human plasminogen to plasmin. *J biol Chem* 242:2333, 1967.
- Robertson, B.R.: Effect of nicotinic acid on fibrinolytic activity in health, in thrombotic disease and in liver cirrhosis. *Acta chir scand* 137:643, 1971.
- Robertson, B.R., Pandolfi, M. & Nilsson, I.M.: Response of local fibrinolytic activity to venous occlusion of arms and legs in healthy volunteers. *Acta chir scand* 138:437, 1972.
- Rodriguez-Erdmann, F. & Guttman, R.D.: Coagulation in renal allograft rejection. *New Engl J Med* 281:1428, 1969.
- Rowley, P.T. & Oette, D.: Characteristics of the anti-trypsin activity of human serum. *J clin Path* 26:48, 1973.
- Salaman, J.: Renal transplant rejection detected with 1125-fibrinogen. *Proc Roy soc Med* 65:476, 1972.
- Salisbury, P.F., Dunn, M.S. & Murphy, E.A.: Apparent free amino acids in deproteinized plasma of normal and uremic persons. *J clin Invest* 36:1227, 1957.
- Salmon, J. & Lamberg, P.H.: Determination of fibrinogen derivatives by immunofluorescence in experimental and clinical kidney disorders. *Scand J Haemat Suppl* 13:351, 1971.
- Sawyer, D., Alkjaersig, N., Fletcher, A.P. & Sherry, S.: Thrombolytic therapy. *Arch intern Med* 107:274, 1961.
- Schmitz, A.: Über die Freilegung von aktivem Trypsin aus Blutplasma. Zweite Mitteilung zur Kenntnis des Plasmatrypsinsystems. *Z Physiol Chem* 250:37, 1937.
- Schreiber, A.D., Kaplan, A.P. & Austen, K.F.: Plasma inhibitors of the components of the fibrinolytic pathway in man. *J clin Invest* 52:1394, 1973.
- Schultze, H.E., Göllner, I., Heide, K., Schönenberger, M. & Schwick, G.: Zur Kenntnis der α -Globuline des menschlichen Normalserums. *Z Naturforsch* 10b:463, 1955.
- Schultze, H.E., Heide, K. & Haupt, H.: α_1 -antitrypsin aus Humanserum. *Klin Wschr* 40:427, 1962.

- Schultze, H.E., Heimbürger, N., Heide, K., Haupt, H., Störiko, K. & Schwick, H.G.: Preparation and characterization of α_1 -trypsin inhibitor and α_2 -plasmin inhibitor of human serum. Proc. 9th Congr. Europ. Soc. Haemat. Lisbon 1963 (S. Karger, Basel/New York), pp 1315-1320, 1963.
- Schwartz, M.L., Pizzo, S.V., Hill, R.L. & McKee, P.A.: The subunit structures of human plasma and platelet factor XIII (fibrin stabilizing factor). J biol Chem 246:5851, 1971.
- Schwartz, M.L., Pizzo, S.V., Hill, R.L. & McKee, P.A.: Human factor XIII from plasma and platelets. J biol Chem 248:1395, 1973.
- Schwick, H.G. & Heimbürger, N.: Biochemie der Fibrinolyse. Thromb Diath haemorrh Suppl 32:9, 1969.
- Schwick, H.G., Heimbürger, N. & Haupt, H.: Antiproteinasen des Humanserums. Z Ges inn Med 21:193, 1966.
- Schönebeck, J., Andersson, L. & Hedner, U.: Isoelectric focusing and Sephadex-filtration of urokinase. A preliminary report. Folia Haemat, Leipzig 95:142, 1971.
- Semeraro, N., Donati, M.B. & Vermylen, J.: Detection of fibrinogen-related antigen in urine with two latex techniques. IV Intern. Congr. Soc. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Shah, B.C., Ambrus, J.L., Mink, I.B., Albert, D.J., Sampson, D. & Murphy, G.P.: Fibrin degradation products in renal parenchymal disease states and renal transplant patients. Transplantation 14:705, 1972.
- Shamash, Y. & Rimon, A.: The plasmin inhibitors of plasma. I. A method for their estimation. Thromb Diath haemorrh 12:119, 1964.
- Sharma, S.D., Chugh, K.S., Singh, G.B. & Chhuttani, P.N.: Fibrinolytic activity of blood in uraemia. Indian J med Res 55:950, 1967.
- Sherry, S.: Fibrinolysis. Ann Rev Med 19:247, 1968.
- Sherry, S., Lindemeyer, R.I., Fletcher, A.P. & Alkjaersig, N.: Studies on enhanced fibrinolytic activity in man. J clin Invest 38:810, 1959.
- Shulman, N.R.: Studies on the inhibition of proteolytic enzymes by serum. III. Physiological aspects of variation in proteolytic inhibition. The concurrence of changes in fibrinogen concentration with changes in trypsin inhibition. J exp Med 95:605, 1952.
- Simon, G. & Chatelanat, F.: Fibrine et fibrinoïde dans les glomérulopathies. Etude comparée en pathologie expérimentale et spontanée. Path Microbiol (Basel) 26:191, 1963.

- Sjoerdsma, A. & Nilsson, I.M.: Aliphatic amino compounds as inhibitors of plasminogen activation. *Proc Soc exp Biol Med* 103:533, 1960.
- Smith, G.F. & Bang, N.K.: Formation of soluble fibrin polymers. Fibrinogen degradation fragments D and E fail to form soluble complexes with fibrin monomer. *Biochemistry* 11:2958, 1972.
- Smyrniotis, F.E., Fletcher, A.P., Alkjaersig, N. & Sherry, S.: Urokinase excretion in health and its alteration in certain disease states. *Thromb Diath haemorrh* 3:257, 1959.
- Solum, N.O., Rigollot, C., Budzynski, A.Z. & Marder, V.J.: A quantitative evaluation of the inhibition of platelet aggregation by low molecular weight degradation products of fibrinogen. *Brit J Haemat* 24:419, 1973.
- Starzl, T., Lerner, R., Dixon, F., Groth, C., Brettschneider, L. & Terasaki, P.: Schwartzman reaction after human renal homotransplantation. *New Engl J Med* 278:642, 1968.
- Stefanini, M., Ewbank, R.L. & Andracki, E.G.: Deficiency of fibrin stabilizing factor: Report of a case probably congenital, with observations on the effects of treatment with ϵ -aminocaproic acid and with prednisolone. *Amer J clin Path* 57:364, 1972.
- Steinbuch, M., Audran, R., Reuge, C. & Blatrix, Ch.: Etude d'une α -globuline contenant du zinc: la protéine π . *Prot Biol Fluids* 14:185, 1966.
- Steinbuch, M. & Blatrix, C.: Action antiprotéase de l' α_2 -macroglobuline. I. Activités antiplasmine et antitrypsine. *Rev franç Etud Clin Biol* 13:142, 1968.
- Steinbuch, M., Blatrix, C., Drouet, J. & Amouch, P.: Study of the α_2 -macroglobulin/plasmin interaction mechanism. *Path Biol Suppl* 20:52, 1972.
- Steinbuch, M. & Loeb, J.: Isolation of an α_2 -globulin from human plasma. *Nature* 192:1196, 1961.
- Steinbuch, M., Quentin, M. & Peioudier, L.: Specific technique for the isolation of human α_2 -macroglobulin. *Nature (Lond)* 205:1225, 1965.
- Stiehm, E.R., Kuplic, L.S. & Uehling, D.T.: Urinary fibrin split products in human renal disease. *J Lab clin Med* 77:843, 1971.
- Stiehm, E.R. & Trygstad, C.W.: Split products of fibrin in human renal disease. *Amer J Med* 46:774, 1969.
- Störiko, K. & Schwick, G.: Die quantitative immunologische Bestimmung des α_1 -Anti-trypsins im menschlichen Serum. In Peeters' *Proc XIth Coll. Protides of the Biol Fluids*, Brügge 1963 (Elsevier, Amsterdam) pp 411-414, 1964.

- Summario, L., Arzadon, A., Bernabe, P., Robbins, K. & Barlow, G.H.: Characterization of the NH₂-terminal glutamic acid and NH₂-terminal lysine forms of human plasminogen isolated by affinity chromatography and isoelectric focusing methods. *J Biol Chem* 248:2984, 1973.
- Summario, L., Hsieh, B. & Robbins, K.C.: The specific mechanism of activation of human plasminogen to plasmin. *J Biol Chem* 242:4279, 1967a.
- Summario, L., Hsieh, B., Groskopf, W.R., Robbins, K. & Barlow, G.H.: The isolation and characterization of the S-carboxymethyl β (light) chain derivative of human plasmin. The localization of the active site on the β (light) chain. *J Biol Chem* 242:5046, 1967b.
- Summario, L., Hsieh, B., Groskopf, W.R. & Robbins, K.C.: Direct activation of human plasminogen by streptokinase. *Proc Soc exp Biol (N Y)* 130:737, 1969.
- Summario, L., Robbins, K.C. & Barlow, G.H.: Isolation and characterization of the S-carboxymethyl heavy chain derived of human plasmin. *J Biol Chem* 246:2143, 1971a.
- Summario, L., Robbins, K.C. & Barlow, G.H.: Dissociation of the equimolar human plasmin-streptokinase complex. Partial characterization of the isolated plasmin and streptokinase moieties. *J Biol Chem* 246:2136, 1971b.
- Sutor, A.H., Schindera, F., Jacobi, H. & Künzer, W.: Haemolytic-uraemic syndrome: thrombocyturia after treatment with streptokinase and aspirin. *Lancet* 2:762, 1972.
- Svanberg, L., Hedner, U. & Åstedt, B.: Value of determinations of FDP during pregnancy using an immunochemical and a latex agglutination inhibition test. *Acta obstet gynec scand* (In press) 1973.
- Takada, A., Takada, Y. & Ambrus, J.L.: Streptokinase-activatable proactivator of human and bovine plasminogen. *J Biol Chem* 245:6389, 1970.
- Takada, A., Takada, Y. & Ambrus, J.L.: Proactivators in the fibrinolysin system. *Thromb Diath haemorrh Suppl* 47:37, 1971.
- Takada, A., Takada, Y. & Ambrus, J.L.: Further studies of plasminogen proactivator. *Biochim Biophys Acta* 263:610, 1972.
- Takada, Y., Takada, A. & Ambrus, J.L.: Comparative study of proactivators of the fibrinolysin system in three mammalian species. *Thromb Diath haemorrh* 21:594, 1969.
- Takagi, T. & Konishi, K.: Purification and some properties of fibrin stabilizing factor. *Biochim Biophys Acta* 271:363, 1972.

- Takeda, Y.: Plasminogen- ^{125}I responses in dogs to a single injection of urokinase and typhoid vaccine and to vascular injury. *J clin Invest* 51:1363, 1972.
- Taylor, Jr., F.B. & Beisswenger, J.G.: Identification of modified streptokinase as the activator of bovine and human plasminogen. *J biol Chem* 248:1127, 1973.
- Taylor, L.M., Slade, C.L., Pizzo, S.V., Hill, R.L. & McKee, P.A.: The equivalence of fragment E from human fibrinogen and fibrin. III Intern. Congr. Soc. Thromb. Haemostasis, Washington D.C., 1972, p 152.
- Teger-Nilsson, A.-C.: Release of human and horse fibrinopeptides. *Acta chem scand* 21:1879, 1967.
- Thorsen, S.: The inhibition of tissue plasminogen activator and urokinase-induced fibrinolysis by some natural proteinase inhibitors and by plasma and serum from normal and pregnant subjects. *Scand J clin Lab Invest* 31:51, 1973.
- Thorsen, S. & Astrup, T.: Biphasic inhibition of urokinase-induced fibrinolysis by E-aminocaproic acid; distinction from tissue plasminogen activator. *Proc Soc exp Biol Med* 130:811, 1969.
- Timpl, R. & Gollwitzer, R.: Cyanogen bromide cleavage of bovine fibrinogen. Identification of a dimeric N-terminal peptide and two other disulfide containing fragments. *FEBS letters* 29:92, 1973.
- Todd, A.S.: The histological localization of plasminogen activator. *J path Bact* 78:281, 1959.
- Todd, A.S.: Endothelium and fibrinolysis. *Atherosclerosis* 15:137, 1972.
- Tomar, R.H. & Taylor, F.B.: The streptokinase-human plasminogen activator complex. *Biochem J* 125:793, 1971.
- Troll, W. & Sherry, S.: The activation of human plasminogen by streptokinase. *J biol Chem* 213:881, 1955.
- Troll, W., Sherry, S. & Wachman, J.: The action of plasmin on synthetic substrates. *J biol Chem* 208:85, 1954.
- Tyler, H.M.: Studies on the activation of purified human factor XIII. *Biochim Biophys Acta* 222:396, 1970.
- Ulutin, S.B. & Aktuglu, G.: The release of platelet antiplasmins. IVth Intern. Congr. Thromb. Haemostasis, Vienna, June 19-22, 1973.
- Uszynski, M. & Abildgaard, U.: Separation and characterization of two fibrinolytic inhibitors from human placenta. *Thromb Diath haemorrh* 25:580, 1971.

- Uszynski, M. & Uszynska-Folejewska, R.: Plasminogen activator and urokinase inhibitor in myometrium, placenta and amniotic fluid. *Amer J Obstet Gynec* 7:1041, 1969.
- Uttley, W.S.: Serum levels of fibrin/fibrinogen degradation products in the haemolytic-uraemic syndrome. *Arch Dis Childh* 45:587, 1970.
- Vassalli, P. & McCluskey, R.T.: The pathogenic role of the coagulation process in rabbit Masugi nephritis. *Amer J Path* 45:653, 1964.
- Vassalli, P. & McCluskey, R.T.: The coagulation process and glomerular disease. *Amer J Med* 39:179, 1965.
- Vassalli, P., Morris, R.H. & McCluskey, R.T.: The pathogenic role of fibrin deposition in the glomerular lesions of texemia of pregnancy. *J exp Med* 118:467, 1963.
- Vermynen, J., Dotremont, G., Donati, M.B., de Gaetano, G. & Michielsen, P.: Pharmacological modification of urinary excretion of fibrinogen-like material in glomerulonephritis. *Scand J Haemat Suppl* 13:365, 1971.
- Vermynen, J., Dotremont, G., Donati, M.B., Molla, A. & Michielsen, P.: Urinary excretion of fibrinogen-fibrin related antigen in glomerulonephritis; effect of Indomethacin. III Intern. Congr. Soc. Thromb. Haemostasis, Washington D.C., Aug 22-26, 1972.
- Vermynen, J., Dotremont, G., de Gaetano, G., Donati, M.B. & Michielsen, P.: Indomethacin and urinary excretion of fibrinogen-like material in proliferative glomerulonephritis. *Europ J clin Biol Res* 15:979, 1970.
- Vignal, A., Blatrix, Ch. & Steinbuch, M.: Fibrinogen free of plasminogen as substrate for fibrinolytic assays. *Nature* 193:693, 1962.
- Vreeken, J., Boomgaard, J. & Deggeller, K.: Urokinase excretion in patients with renal diseases. *Acta med scand* 180:153, 1966.
- Wallén, P.: Plasminic degradation of fibrinogen. *Scand J Haemat Suppl* 13:3, 1971.
- Wallén, P. & Bergström, K.: Purification of human plasminogen on DEAE-cellulose. *Acta chem scand* 14:217, 1960.
- Wallén, P. & Wiman, B.: Characterization of human plasminogen I. On the relationship between different molecular forms of plasminogen demonstrated in plasma and found in purified preparations. *Biochim Biophys Acta* 221:20, 1970.

- Wallén, P. & Wiman, B.: Characterization of human plasminogen. II. Separation and partial characterization of different molecular forms of human plasminogen. *Biochem Biophys Acta* 257:122, 1972.
- Walls, W.D. & Losowsky, M.S.: Plasma fibrin stabilizing factor (FSF) activity in normal subjects and patients with chronic liver disease. *Thromb Diath haemorrh* 21:134, 1969.
- Wardle, E.N., Menon, I.S. & Rastogi, S.P.: Study of proteins and fibrinolysis in patients with glomerulonephritis. *Brit med J* 2:260, 1970.
- Wardle, E.N. & Taylor, G.: Fibrin breakdown products and fibrinolysis in renal disease. *J clin Path* 21:140, 1968.
- Wardle, E.N. & Uldall, P.R.: Effect of heparin on renal function in patients with oliguria. *Brit med J* 4:135, 1972.
- Warren, B.A.: Fibrinolytic properties of vascular endothelium. *Brit J exp Path* 44:365, 1963.
- Wasserman, A.E.: Streptokinase activation of a proteolytic enzyme in human blood. *Arch Biochem Biophys* 41:158, 1952.
- Weiner, M., Redisch, W. & Steele, J.M.: Occurrence of fibrinolytic activity following administration of nicotinic acid. *Proc Soc exp Biol Med (N Y)* 98:755, 1958.
- Weiss, W.A.: Normalwerte spezifischer Serumproteine im Kindes- und Erwachsenen alter. *Klin Wschr* 43:273, 1965.
- Whitaker, J.R.: Determination of molecular weights of proteins by gel filtration on Sephadex. *Anal Chem* 35:1950, 1963.
- White, W.F., Barlow, G.H. & Mozen, M.M.: The isolation and characterization of plasminogen activators (urokinase) from human urine. *Biochemistry* 5:2160, 1966.
- Wilson, C.B.: Immunological mechanisms of glomerulonephritis. *Calif Med* 116:47, 1972.
- Wiman, B.: On the structure of the N-terminal fragment of human plasminogen obtained after cleavage with cyanogen bromide. *Thrombosis Research* 1:89, 1972.
- Wiman, B. & Wallén, P.: Activation of human plasminogen by an insoluble derivative of urokinase. *Europ J Biochem* 36:25, 1973.
- Wolf, P.: A modification for routine laboratory use of Stefanini's method of estimating factor V activity in human oxalated plasma. *J clin Path* 6:34, 1953.

- Wünschmann-Henderson, B., Horwitz, D.L. & Astrup, T.: Release of plasminogen activator from viable leukocytes of man, baboon, dog and rabbit. *Proc Soc exp Biol Med* 141:634, 1972.
- Yatzidis, H.: Evidence of fibrinogenuria in the nephrotic syndrome. *Nature* 201:187, 1964.
- Zühlke, V. & Hermann, G.: Immunologische Untersuchungen der Proteinurie während Abstossungskrisen nach allogenen Nierentransplantationen beim Menschen. *Klin Wschr* 4:201, 1970.
- Åstedt, B. & Pandolfi, M.: Ontogenesis of tissue plasminogen activator in the human. *Thromb Diath haemorrh* 25:469, 1971.
- Åstedt, B. & Pandolfi, M.: On release and synthesis of fibrinolytic activators in human organ culture. *Rev Europ Etudes Clin Biol* 17:261, 1972.
- Åstedt, B., Pandolfi, M. & Nilsson, I.M.: Inhibitory effect of placenta on plasminogen activation in human organ culture. *Proc Soc exp Biol Med* 139:1421, 1972.

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